

UNDERSTANDING THE RELATIONSHIPS BETWEEN PLANT COMMUNITIES, FISH
COMMUNITIES, AND SEDIMENT CHEMISTRY IN PRISTINE SHALLOW LAKE
WETLANDS, RED LAKE, MN

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ABSTRACT

Over 90% wetland loss in many areas of the United States has triggered extreme concern for declining biodiversity and loss of ecosystem services. Studies assessing ecological characteristics of unaltered wetlands are critical to interpreting potential responses to anthropogenic changes and how ecosystem characteristics and services might respond to future climate dynamics. Previous research shows fish and plant communities play an important role in shallow lake ecology by creating stability within the clear state. Understanding these mechanisms in a pristine setting is important for successful conservation and restoration. Our objectives, part of a long term monitoring study (2009-2015), were 1) Evaluate patterns in multi-element concentrations of pore water along a gradient from upland areas through emergent vegetation to the open water of shallow lake wetlands 2) Assess seasonal changes of multi-element concentrations of pore water during the growing season of shallow lake wetlands 3) Measure the influence of fish communities species composition on plant communities species composition 4) Evaluate how sediment chemistry impacts plant communities. We evaluated pore water chemistry on two lakes using dialysis vials from June-October 2014. We selected 24 shallow lakes located within Red Lake Nation Indian Reservation, MN, USA to conduct the remaining objectives. At each lake we assessed fish community structure and abundance using fyke nets and experimental gill nets for a 24-hr period during July. Aquatic and emergent vegetation was assessed simultaneously by rake throws and Daubenmire frame, and sediment samples were collected by taking a surface grab sample. Sediment samples were taken in the open water and emergent vegetation zones. Pore water did not change across a gradient from upland to open water, but did change over the season from June to October. We found fish community composition did not change significantly from 2009 to 2015, but fish biomass significantly decreased from 2009 to 2015. No species-specific relationships between vegetation

and fish existed, but species specific relationships existed between vegetation and sediment characteristics in both the open water and emergent vegetation zones. Results will be used as reference points for restoration and conservation, and serve for a better understanding of shallow lake state stability.

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LIST OF ABBREVIATIONS

Chl <i>a</i>	Chlorophyll a
CRM.....	Certified reference material
ICP-OES	Inductively coupled plasma optical emissions spectrophotometer
ICP-MS	Inductively coupled plasma mass spectrometry
SAV.....	Submerged aquatic vegetation.

CHAPTER 1: GENERAL INTRODUCTION

Introduction

Extensive amounts of wetland losses throughout the United States, greater than 90% in some areas (Mitsch and Gosselink, 2007), have caused concern about loss of biodiversity and ecosystem services (Bayley et al., 2013; Brothers et al., 2013; Hobbs et al., 2014; Marton et al., 2015). Wetlands provide important ecosystem services including nesting and brood rearing habitat for waterfowl species (Bouffard and Hanson, 1997; Perrow et al., 1997), important stopover sites along migration routes for resting and replenishing energy reserves (Bookhout et al., 1989), flood mitigation and water quality improvement (Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007), and wetlands are culturally and economically important to Native American tribes for cultivating wild rice (*Zizania palustris.*) (Treuer, 2010). Isolated wetlands are extremely important on the landscape because they often have longer water residence time when compared with connected wetlands, which increases water quality by enabling particulates and contaminants to settle out of the water column or be incorporated into plant tissues (Whigham and Jordan, 2003; Marton et al., 2015). They can also significantly reduce water flow, thus buffering flooding events, making them valuable on the landscape for improving quality of human life (Carpenter and Lodge, 1986; Whigham and Jordan, 2003; Mitsch and Gosselink, 2007; Marton et al., 2015). Wetland loss throughout the United States has been due to anthropogenic influences such as farming and building development, but even those wetlands that are not lost can still be altered, which can reduce their biodiversity, ecosystem services, and value (Lougheed et al., 2001; Whigham and Jordan, 2003; Mitsch and Gosselink, 2007; Kissoon et al., 2013; Kissoon et al., 2015).

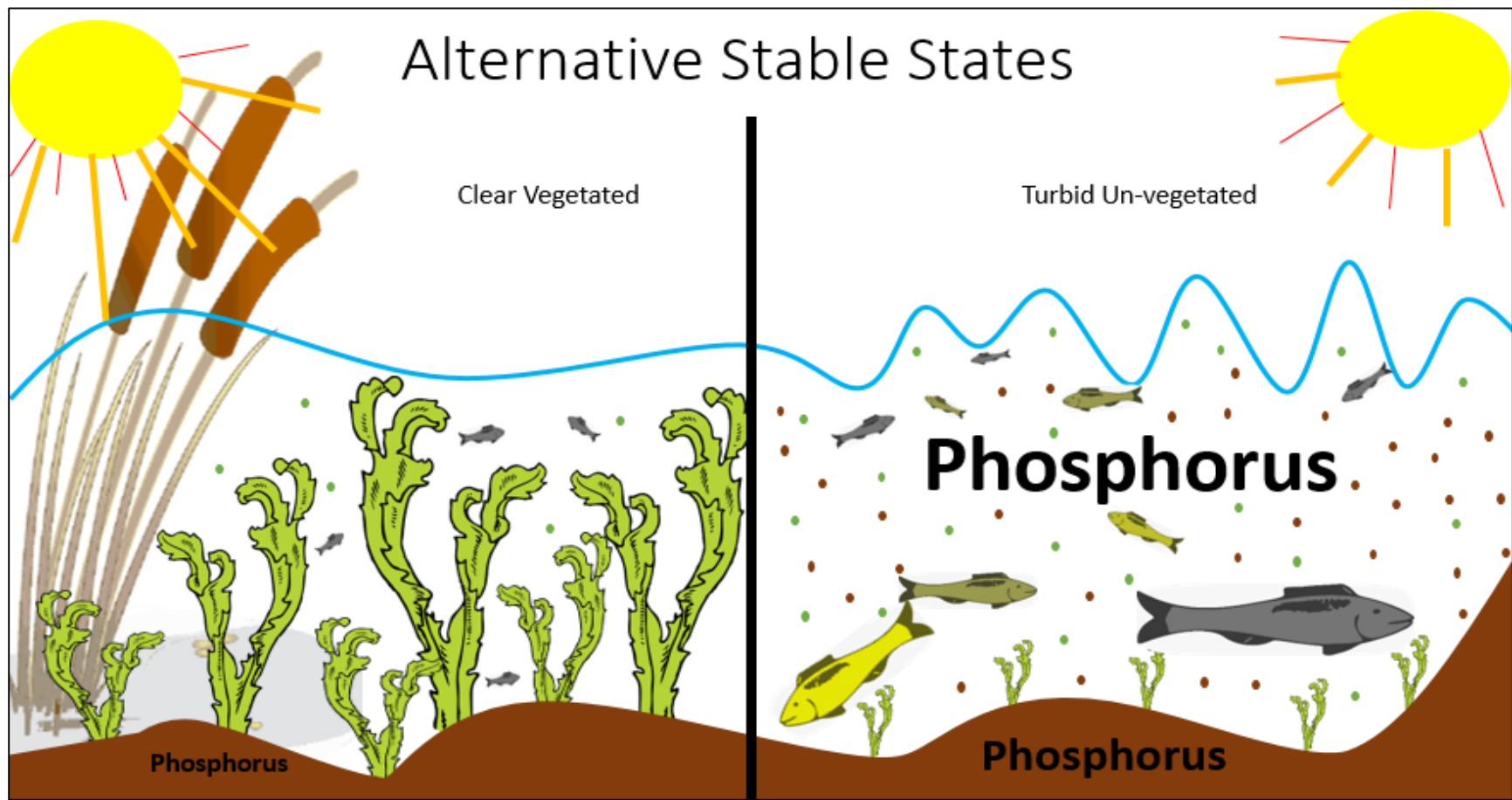
Understanding conditions present in pristine wetlands could make future conservation and restoration projects more successful by better understanding how human influence has altered wetlands in an anthropogenic setting. Many mitigation projects have failed because environmental characteristics did not accurately mimic those of the naturally occurring wetlands, and desired plant communities failed to establish (Stolt et al., 2000; Seabloom and van der Valk, 2003a; Seabloom and van der Valk, 2003b; Stapanian et al., 2013; Hobbs et al., 2014; Sullivan et al., 2014). By studying pristine wetlands, those having been least impacted by humans, a better understanding of naturally occurring soil and sediment characteristics, and plant communities, can help managers choose more appropriate mitigation or restoration sites to better represent natural conditions and achieve successful results. Understanding the ecology of pristine wetlands is also important for conservation in the face of climate change and potential impacts to existing wetlands. Each year state and federal agencies along with private organizations spend millions of dollars on wetland conservation, restoration, and research (USDA, 2013), so with a better understanding of pristine wetland conditions could come more effective spending, saving agencies and organizations money.

Alternative Stable States

Shallow lake wetlands, are of specific concern because they are unique aquatic systems in that, shallow lakes are typically dominated by densely colonized macrophytes, and do not experience the thermal stratification that deeper lakes do (Scheffer, 2004). This means shallow lakes do not have the same characteristics of deep lakes in terms of fish communities, plant communities, and sediment characteristics. The term shallow lake refers to a lake less than 3 m deep (not restricted in surface area size) and the surrounding area of inundated soils and characteristic emergent wetland plant species (Scheffer, 2004). Macrophyte colonization and the

fact that the water and sediment interact extensively make shallow lakes function quite differently than deep lakes or wetland types, which undergo wet to dry periods (Scheffer, 2004; Wezel et al., 2013). Shallow lake wetlands are also unique in that they can exhibit alternative stable states, changing from a clear vegetated state to a turbid un-vegetated state, but previous research has yet to uncover the complete process and causation of this shift (Scheffer et al., 2001; Bayley and Prather, 2003; Scheffer, 2004; Van Geest et al., 2005; Bayley et al., 2007; Zimmer et al., 2009; Hobbs et al., 2012). Jackson's (2003) study revealed that the state of a shallow lake is probably due to interactions within each lake and the immediate surrounding area, and is not an effect of region or latitude because shallow lakes within the same region exhibit both the clear and the turbid state, even when they are impacted by the same climatic effects and land use practices. Once a shallow lake is in a state, it tends to stay that way because of positive feedback mechanisms, and often will not change back to the original state at the same original conditions. For example, if nutrient loading on a lake caused a shift from clear to turbid, it may take lower than the original nutrient levels for the lake to change from turbid back to clear (Scheffer et al., 2001; Hobbs et al., 2012). The clear vegetated state is stable because plant communities take up nutrients, which decreases the nutrient availability in the water column, providing fewer resources for phytoplankton and algae so algal blooms do not occur, and water clarity is kept high (Cronk and Fennessy, 2001; Scheffer, 2004). Macrophytes also provide hiding cover and habitat for zooplankton such as *Daphnia* spp., which feed on algae and phytoplankton, which helps to keep the water column clear, also providing more sunlight and energy for macrophyte growth (Bayley and Prather, 2003; Scheffer, 2004). Without macrophytes, zooplankton do not have adequate habitat for high survival rates and populations can decrease as predation increases from fish. The lower the population abundance of zooplankton falls, the higher the algal and

phytoplankton populations can increase because of that lack of predation (Sørensen et al., 2001; Villena and Romo, 2007). With the removal of macrophytes in a lake, sediment can become re-suspended more readily by wind energy and wave action, because the plants are not there to absorb that energy (Engel and Nichols, 1994; Scheffer et al., 2001; Bayley et al., 2007). Benthivorous fish can also stir up sediment decreasing water clarity as they feed (Zimmer et al., 2003; Scheffer, 2004; Roozen et al., 2007). Human activities, such as plant removal for recreation and nutrient loading from human and agricultural development can cause the shift from clear vegetated to turbid un-vegetated shallow lakes (Scheffer, 2004; Bayley et al., 2013), but some lakes with naturally nutrient rich soils in areas absent from human development have undergone state switches, indicating perhaps in some areas these states are not all that stable after all (Bayley and Prather, 2003; Bayley et al., 2007) (fig.1.1).



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Figure 1.1: Infographic representing the characteristics of alternative stable states. On the left side is the clear vegetated state, with dense macrophytes which take up nutrients in the water column and out-compete much of the algae which keeps the water column clear. Lower fish biomass means less feeding pressure on zooplankton which in turn means more feeding pressure on phytoplankton. Macrophytes absorb wind energy and reduce resuspension of sediment particulates in the water column. A clear water column also means greater light penetration which is necessary for plant growth, as depicted on the left. The right side depicts the turbid state. Greater amounts of nutrients in the water column, such as phosphorus, lead to algal blooms. Large fish communities can re-suspend sediment particles decreasing water clarity, which in turn decreases light penetration and macrophyte growth. Without macrophytes, wind creates waves which re-suspend sediment particulates and create a more turbid water column.

Fish Communities

Fish community composition and trophic interactions have been well documented in shallow lake wetlands and may contribute to their stability in the turbid state (O'Brien, 1979; Hanson and Butler, 1994a; Hanson and Butler, 1994b; Jeppesen et al., 1999; Zimmer et al., 2001; Zimmer et al., 2002; Zimmer et al., 2003; Hanson et al., 2005; Potthoff et al., 2008; Herwig et al., 2010; Friederichs et al., 2011; Nolby et al., 2015). Fish biomass and community composition are likely factors contributing to the stability of shallow lake state (Norlin et al., 2006; Roozen et al., 2007; Nolby et al., 2015), and those lakes which are interconnected and larger in size have a greater chance for fish colonization and to be in the turbid state (Herwig et al., 2010).

Zooplankton are also important components of shallow lake ecology and food webs, and directly related to fish communities and state of shallow lakes (Jeppesen et al., 1997; Sørensen et al., 2001; Zimmer et al., 2001; Laurich et al., 2003; Norlin et al., 2006). Zooplankton are prey for fish communities, and are also grazers on phytoplankton (O'Brien, 1979; Scheffer, 2004). Zooplankton have evolved to have defenses against their predators, but are still important food sources for many fish species, and in many cases increases in fish abundance leads to a decrease in zooplankton abundance and body size (O'Brien, 1979; Hanson and Butler, 1994a; Zimmer et al., 2001; Zimmer et al., 2003). Larger zooplankton are more effective grazers on phytoplankton than smaller zooplankton, so decreases in zooplankton body size could lead to decreased grazing pressure on phytoplankton and increased water turbidity (O'Brien, 1979). *Daphnia* spp. are large bodied zooplankton and are also effective grazers on phytoplankton, but their abundance is negatively associated with fish abundance, probably due to their large body size making them easily seen by predators (O'Brien, 1979; Sørensen et al., 2001). Shallow lakes may make

zooplankton communities especially susceptible to fish predation because they cannot escape predation by vertical migration, and are therefore always interacting with their predators, increasing their chances of visibility and predation events (O'Brien, 1979).

The presence of fish communities has also been linked to lower abundance of submerged aquatic vegetation (SAV), increased phytoplankton, and increased turbidity in shallow lakes (Hanson and Butler, 1994b; Zimmer et al., 2001; Zimmer et al., 2002; Hanson et al., 2005). Zimmer et al. (2001) reported that *Pimephales promelas* (fathead minnows), can be linked to decreases in large bodied zooplankton, while chlorophyll *a*, turbidity, and total phosphorus increased with the colonization of fathead minnows. Jeppesen et al. (1999) found similar results, with turbid lakes having higher fish biomass than clear lakes, which was then associated with higher chlorophyll *a*, turbidity, and decreased macrophyte abundance. The turbid lakes were dominated by planktivorous fish species, while the clear lakes had greater piscivorous fish abundance, which may indicate that fish community composition, and thus food web interactions, can play a role in the state stability of shallow lakes. Sørensen et al. (2001) and Villena and Romo (2007) also found a negative relationship between zooplankton and fish abundance, again indicating a direct relationship and food web interactions between fish and zooplankton, which then leads to cascading effects to phytoplankton, chlorophyll *a*, and turbidity.

Fish community composition also impacts shallow lake state stability, not solely the presence or absence of fish. Those lakes dominated by benthivorous fish species are more likely to be turbid, because as benthivorous fish feed, they resuspend sediment particles into the water column, making essential nutrients more available to phytoplankton, enabling increases in phytoplankton abundance and therefore turbidity (Zimmer et al., 2001; Roozen et al., 2007).

Benthivorous fish can also affect species composition of algae within shallow lakes. In the absence of benthivorous fish, a higher percentage of mobile phytoplankton were present, while in the presence of benthivorous fish more green algae and diatoms were seen. This is probably due to the fact that as benthivorous fish feed they help resuspend those phytoplankton which are non-mobile (diatoms and green algae), back into the water column after they have settled onto the sediment surface of shallow lakes (Roozen et al., 2007).

Changing food webs by adding a top predator, piscivorous fish species, does not seem to be a viable long term solution for increasing lake clarity (Potthoff et al., 2008). By adding a top piscivore predator to shallow lake systems, one would expect to see decreases in planktivorous fish species resulting in increases in zooplankton, then therefore decreases in phytoplankton, which would ultimately increase water clarity. Although Potthoff et al. (2008) found that adding young walleye fry (*Sander vitreus*), did decrease planktivorous fish abundance initially, this effect was not long term, and additional stocking may be needed to continue to observe lower planktivore abundances. They did also see greater macrophyte abundance, greater abundance of large bodied invertebrates, and decreased phytoplankton abundance. These were not significant in the study period, which may suggest the need for repeated stocking events in order for significant changes in SAV or zooplankton to occur. Or it may suggest a lag time for these changes to take place and establish at significantly higher levels than before the introduction. Reducing planktivores can lead to a small trend towards clearer lakes, indicating that planktivorous fish species may be associated with a trend towards the turbid state in shallow lakes (Potthoff et al. 2008). Friederichs et al.'s (2011) study evaluated piscivore abundance, and found no relationship between phytoplankton and piscivore abundance, which somewhat contradicts Potthoff et al.'s (2008) results, but may be due to different sampling sites and lake

conditions. Friederich et al.'s (2011) study though did find a shift from soft rayed planktivores to spiny rayed planktivores, indicating those species with better defenses against predation persisted, and may still have had a large effect on zooplankton abundance. The presence of spiny rayed planktivores which would in turn affect phytoplankton abundance and may be why they didn't see a relationship between piscivores and phytoplankton, as the fish species that were not predated upon were still exerting a strong effect on zooplankton populations.

Fish communities not only affect zooplankton communities but can also affect waterfowl communities (Epnors et al., 2010). Fish communities compete with waterfowl for food sources such as zooplankton and aquatic invertebrates, which can lead to increased turbidity and decreased waterfowl. This is less desirable when managing for waterfowl (Hanson and Butler, 1994b). When lake size is controlled for in the data analysis, lakes with small bodied fish or no fish at all have significantly greater waterfowl use for breeding and molting birds than those lakes with larger fish (Epnors et al., 2010), perhaps due to competition for resources.

Although many studies have documented food web interactions with fish, zooplankton, and SAV, few studies have done so in a pristine setting (Bayley et al., 2009; Jackson, 2003), and it is poorly understood if species specific relationships between fish and SAV are occurring in the ecology of shallow lakes, contributing to state stability. Understanding fish communities in pristine wetlands is important, because it may lead to a better understanding of how these ecosystems function in their original conditions. Understanding fish communities will also lead to understanding how they influence other ecological characteristics, which will help with management decision making, and lead to better restoration and conservation efforts.

Plant Communities

SAV is also a very important component of shallow lake ecology, and understanding how SAV contributes to the alternative stable state theory, and shallow lake ecology in a pristine setting will also lead to better natural resource management and restoration efforts. SAV contributes to the stability of the clear state in many ways including reducing sediment re-suspension by absorbing wave energy, providing refuge for zooplankton communities from predators, and inhibiting phytoplankton growth by the production of allelopathic chemicals and releasing them into the water column (Engel and Nichols, 1994; Jackson, 2003; Scheffer, 2004; de Vicente et al., 2006; Mulderij et al., 2007; Villena and Romo, 2007; Hilt and Gross, 2008).

The presence of SAV and emergent vegetation can help stabilize sediment particles on shallow lake bottoms, increasing the clarity of the water, and helping stabilize the clear, plant-dominated state (Engel and Nichols, 1994; Horppila and Nurminen, 2003; Scheffer, 2004; de Vincente et al., 2006; Sullivan et al., 2014). Wind energy creates wave action in the water column of lakes. In shallow lake wetlands wave action stir sediment and suspends sediment particles, which then decreases the clarity of the water and reduces light penetration (Jackson, 2003; Scheffer, 2004). Light is essential for plant growth, therefore less light reduces the amount of energy available for plant growth and the end result is a decrease SAV biomass production (Cronk and Fennessy, 2001). De Vincente et al. (2006) compared two shallow lakes, one in the turbid state and one in the clear state, to determine the cause of their differences. They found that the turbid lake was more susceptible to wind and wave action in extreme rain events which caused for continuous mixing of the water column and increased turbidity. The clear lake had extensive SAV growth and biomass and was less susceptible to mixing by wave action than the turbid lake. The SAV absorbs energy from the waves, the plants themselves move, reducing the

amount of energy which reaches the sediment, which reduces the amount of sediment that is stirred into the water column (van Donk and van de Bunk, 2002; Scheffer, 2004). Plant roots in the sediment also help to secure the sediment in place and helps reduce resuspension of particles (Cronk and Fennessy, 2001). The fewer the particulates in the water column the lower the turbidity (Roozen et al., 2007) and greater light penetration, which enables greater SAV biomass production, which then in turn helps stabilize shallow lakes in the clear state (Perrow et al., 1999; Horppila and Nurminen, 2003; Jackson, 2003; Scheffer et al., 2004; Hilt and Gross, 2008).

Suspended sediment particles can also have the attachment of phosphorus, making phosphorus more available in the water column to phytoplankton (Horppila and Nurminen, 2003). Increased phosphorus in the water column can promote phytoplankton growth, which then increases water turbidity, decreasing light penetration and SAV growth (Horppila and Nurminen, 2003; Scheffer, 2004). At low nutrient levels SAV outcompete phytoplankton for essential nutrients (phosphorus and nitrogen), which keeps the water column clearer and lower in turbidity measurements (Zimmer et al., 2003; Kosten et al., 2009). SAV can obtain nutrients from the sediment and water column (Cronk and Fennessy, 2001), which may be an explanation for why they are superior competitors than phytoplankton for nutrients, and can help stabilize the clear state at low nutrient levels (Jeppesen et al., 1999; van Donk and van de Bund, 2002; Zimmer et al., 2003; Kosten et al., 2009; Zimmer et al., 2009; Sayer et al., 2010). This may depend upon the species of SAV present, however. Abundance of *Chara* species is often negatively correlated with phosphorus concentrations, indicating that *Chara* take up and utilize available phosphorus, leaving less available for phytoplankton use (Zimmer et al., 2003). *Potamogeton* species, conversely, did not show any significant relationships with nutrient availability (van Donk and van de Bunk, 2002; Zimmer et al., 2003; Mulderij et al., 2007). Phosphorus is an essential

nutrient for the growth and reproduction of primary producers (Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007), so less available phosphorus in the water column would lead to decreases in abundance of phytoplankton. Even though SAV are better competitors for nutrients, as nutrients levels increase there tends to be a shift from the clear state to the turbid state, and a loss of SAV (van Donk and van de Bund, 2002; Scheffer, 2004; Villena and Romo, 2007; Zimmer et al., 2009; Sayer et al., 2010). This may be because as nutrient levels increase the rate at which SAV can take up nutrients becomes satiated, leaving excess nutrients in the water column that are then available for phytoplankton use. As the phytoplankton use the nutrients they grow and reproduce within the water column, increasing water turbidity and shading out the SAV, leading to a decrease in SAV abundance and biomass production. There seems to be a threshold at which SAV nutrient uptake is satiated, although there is variation in this threshold probably attributed to specific lake characteristics and relationships. Somewhere around $60 \mu\text{gL}^{-1}$ of total phosphorus causes a shift from clear to turbid, although state shift can be seen at much lower concentrations in some lakes (Bayley and Prather, 2003; Bayley et al., 2007; Kosten et al., 2009; Zimmer et al., 2009).

Macrophytes not only stabilize the clear state by reducing sediment resuspension and nutrient availability, but they also provide hiding cover for zooplankton from fish predation (Perrow et al., 1999; Zimmer et al., 2003; Scheffer, 2004; Villena and Romo, 2007; Louette et al., 2009). Understanding how macrophytes contribute to zooplankton populations and abundance is important because zooplankton prey upon phytoplankton and significantly reduce phytoplankton populations, which then causes a decrease in water turbidity (Jeppesen et al., 1999; Scheffer, 2004). *Daphnia* species are some of the most effective grazers on phytoplankton, but are also preferred food sources of planktivorous fish species (especially

fathead minnows and brook sticklebacks), but the addition of macrophytes to shallow lakes provides hiding cover from predatory fish, and there is often a positive relationship with SAV and zooplankton abundances (O'Brien, 1979; Perrow et al., 1999; Laurich et al., 2003; Zimmer et al., 2003). Perrow et al. (1999) found in the presence of fish, the use of SAV significantly increased by all species of *cladocerans*, indicating that zooplankton do use SAV for hiding cover in the presence of fish. They also found the effect of SAV as hiding cover for zooplankton to be significant only when fish densities were lower than 0.2 fish per meter². When densities of fish increased above 0.2 fish per meter², significant reductions in zooplankton populations still resulted from fish predation, indicating the refuge effect of SAV for zooplankton does not exist when fish densities exceed 0.2 fish per meter². This may be because some zooplankton species use SAV as a daytime refuge and graze on phytoplankton at night in the open water, but some planktivorous fish species still feed at night and prey upon the zooplankton in the open water. Other fish species such as *Esox lucius* (northern pike) and *Perca flavescens* (yellow perch) use SAV as cover against predators when they are young and small, therefore they are still able to feed upon the zooplankton in those areas (Perrow et al., 1999). Villena and Romo (2007) also found that in the absence of SAV, the zooplankton : phytoplankton ratio significantly decreased, which was attributed to fish predation, indicating SAV had a refuge effect on zooplankton in this study.

The presence of SAV and hiding cover for zooplankton is an important mechanism for stabilizing shallow lakes in the clear state, but not the only mechanism for stability. Some SAV species can contribute to the stability of the clear state by releasing allelopathic chemicals, which inhibit phytoplankton growth and reproduction, increasing water clarity, and therefore SAV growth (van Donk and van de Bund, 2002; Mulderij et al., 2007; Hilt and Gross, 2008). It is

often hard to measure the direct influences of allelopathy on phytoplankton because many other environmental factors may contribute to macrophyte-phytoplankton competition including species specific interactions; shading, nutrients, and turbidity (Hilt and Gross, 2008; Hilt and Lombardo, 2010). Different species of SAV exert different allelopathic chemical effects. *Cyperus*, *Eleocharis*, *Nelumbo*, *Nymphaea*, *Nuphar*, *Polygonum*, *Myriophyllum*, and *Vallisneria*. Specifically *Myriophyllum spicatum* and *Ceratophyllum demersum*, are known to produce abundant allelopathic chemicals, while *Elodea* and *Chara* species have a marginal allelopathic effect. In contrast *Potamogeton* species are not known to have an allelopathic effects on phytoplankton (Cronk and Fennessy, 2001; Hilt and Gross, 2008). Allelopathic substances are difficult to detect in the water column because they are rapidly transformed by light and bacteria (Cronk and Fennessy, 2001; Hilt and Gross, 2008). Hilt and Gross (2008) found that plants secrete allelopathic substances under a variety of conditions at a variety of levels, and thought stress could be a trigger for allelopathic chemical production, but it was difficult to make clear conclusions as to what would trigger production. Competition for light and resources may be triggers for allelopathic chemical production, but not in all cases (Gopal and Goel, 1993; Cronk and Fennessy, 2001). Mulderij et al. (2007) found *Chara* species and *Stratiotes aloides* secrete allelopathic chemicals, but the shading of floating leaves and reduced sediment resuspension were more effective in reducing phytoplankton abundances than allelopathic chemicals. Allelopathic chemicals may disrupt enzyme production in phytoplankton, reducing their survivability, but the mechanisms of how allelopathy works is still poorly understood and chemical presence is not well documented in the water column, but only in plant materials such as roots and leaves (Cronk and Fennessy, 2001; van Donk and van de Bund, 2002; Mulderij et al., 2007; Hilt and Gross, 2008; Hilt and Lombardo, 2010).

Allelopathic chemical production is therefore probably not a sole factor contributing to clear state stability of shallow lakes, but may contribute to clear state stability when compounded with hiding cover for zooplankton, decreased resources for phytoplankton, and decreased sediment suspension in the water column, though more research is needed for a better explanation of allelopathic chemical interactions with phytoplankton and how they contribute to SAV growth or water clarity. Understanding which species produce allelopathic chemicals may lead to a better understanding of shallow lake state stability when these species are present, and is important to note as it may contribute to shallow lake state stability. Many factors affect SAV community composition, and SAV affects many ecological characteristics, but it is still unclear if species specific interactions play a major role in state stability. Understanding how fish species affect SAV species present in shallow lakes may lead to a more complete understanding of shallow lake ecology, and is an area in need of future research.

Sediment Characteristics

Understanding SAV community composition is important in the understanding of current state conditions, but many factors contribute to SAV community composition including current state stability, environmental factors (lake depth, precipitation, wave action, organic matter accumulation, land use) (Engel and Nicholas, 1994; Bayley et al., 2007; KISSOON et al., 2013), and abiotic factors (pH, alkalinity, salinity, turbidity, nutrients, sediment particle size, and organic matter) (Stewart and Kantrud, 1972; Cowardin et al., 1979; Cronk and Fennessy, 2001; Reddy and DeLaune, 2008; KISSOON et al., 2015). The abiotic factors that make up sediment chemistry are very important in terms of contributing to plant community composition, and may largely be influenced by ground water and parent rock material (Moyle, 1945; Swartz and Gallup, 1978; Cowardin et al., 1979; Barko et al., 1991; Cronk and Fennessy, 2001; Lougheed et

al., 2001; Hansel-Welch, 2003; Capers et al., 2009; Alahuhta et al., 2012). Plants rooted in the sediment of shallow lakes obtain most of their nutrients from the sediment, so sediment characteristics and chemistry contribute largely to the abundance and biomass of vegetation supported and the species which can grow under those conditions (Barko et al., 1999; Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007). Water quality characteristics are also important and can influence shallow lake plant communities. Shallow lakes are shallow there is usually continual mixing of the water, which increases nutrient exchange between the sediment and water column, and benthic invertebrates mix the sediment surface and water with decomposition processes, so the water and sediment of shallow lakes are interconnected (Carpenter and Lodge, 1986; Bark et al., 1991; Scheffer, 2004). Plant communities can also change sediment characteristics by extracting nutrients during growth and reproduction, and returning nutrients to the sediment during senescence and decay (Carpenter and Lodge, 1986; Barko et al., 1991; Cronk and Fennessy, 2001).

Turbidity of the water column can be reduced or influenced by the presence of SAV, but turbidity can also influence the SAV species present (Engel and Nichols, 1994; Hansel-Welch et al., 2002; Scheffer, 2004). In a study observing Lake Christina (located in Minnesota, USA), where the fish community was extirpated in an effort to increase water clarity and to return the lake to the clear-vegetated state from the turbid state, researchers found, during pre-treatment observations, only *Stuckenia pectinata* and *Najas* species present in turbid conditions, while *Najas* species, *Myriophyllum sibiricum* and *Rupia maritima* were found immediately following fish extirpation, indicating these species might be tolerant of slightly turbid conditions. *Chara vulgaris*, *Chara canescens* and *Potamogeton pussilus* were only found after the clear state was returned indicating these species are not tolerant of turbidity and probably need higher light

penetration for survival (Hansel-Welch et al., 2003). Lougheed et al. (2001) found *Elodea canadensis* and *Ceratophyllum demersum* to be indicative of turbid waters while *Pontederia cordata*, *Sparganium* species, *Potamogeton richarsonii*, *Najas flexilis*, *Utricularia* species, and *Potamogeton natans* to be present in clear waters. The discrepancy between species composition and state in these two studies may be because of geological features in the landscape, such as parent rock material, land use, impacting water, and sediment characteristics (Sahuquillo et al., 2012; Kisson et al., 2013; Wezel et al., 2013; Kisson et al., 2015). Capers et al. (2009) found SAV community composition to be very diverse across the landscape with almost 50% of the species observed in shallow lakes occurring in less than five lakes (98 lakes in total surveyed), indicating that plant species can be very sensitive to environmental conditions with water clarity, water pH, lake area, and lake depth, all contributing to plant community assemblage.

The pH in the water column or sediment of shallow lakes can influence plant communities by influencing nutrient dynamics, and chemical composition (Cowardin et al., 1979; Cronk and Fennessy, 2001, Reddy and DeLaune, 2008). Wu et al. (2014) found phosphorus release from the sediment to be highest at alkaline pH values, lowest at a pH of 7, and intermediate at acidic pH values. Phosphorus is essential for plant growth (Cronk and Fennessy, 2001), but at high concentrations can promote phytoplankton and algal blooms, which can shade out SAV communities (van Donk and van de Bund, 2002; Scheffer, 2004; Villena and Romo, 2007; Zimmer et al., 2009; Sayer et al., 2010). This indicates alkaline lakes could have a tendency to be more turbid with lower SAV abundances or biomass production than neutral or acidic lakes. The pH can also influence sediment chemistry because hydrogen and hydroxide ions bind with other ions in sediment or cause redox reactions which can affect nutrient availability and biological processes of plants (Reddy and DeLaune, 2008). Some wetland and

aquatic plant species may be able to tolerate elevated pH, including *Potamogeton crispus*, *Potamogeton illinoensis*, *Potamogeton richarsonii*, and *Stuckenia pectinata* to name a few (Moyle, 1945; Capers et al., 2009; KISSOON et al., 2013). Fewer plants tolerate low pH conditions (Moyle, 1945; Capers et al., 2009), while most wetland and aquatic plant species prefer neutral pH conditions (Moyle, 1945).

The dissolved oxygen content and temperature of the water and sediment also influences phosphorus release and available nutrients to plants, with aerobic conditions having higher phosphorus concentrations than anaerobic conditions, and increases in temperature also influence phosphorus release (Wu et al., 2014). Increased bacteria and benthivorous invertebrate activity, breaking down organic matter and releasing bound up nutrients from decaying materials (Carpenter and Lodge, 1986; Barko et al., 1991; Cronk and Fennessy, 2001). High organic matter content in shallow lake sediments can also limit SAV growth and impact community composition (Barko et al., 1991; KISSOON et al., 2013). High organic matter sediments are usually nutrient poor, because much of those nutrients are bound up within the organic matter and have yet to be released, and those soils with high organic matter content usually have higher abundances of floating leaf and emergent macrophytes (Barko et al., 1991). KISSOON et al. (2013) found *Chara* species, *Utricularia vulgaris*, and *Najas flexilis* to be most common in shallow lakes with high organic matter content, indicating these species might be more tolerant of low nutrient levels.

The surrounding landscape also impacts shallow lake plant communities by influencing runoff rates, nutrient depositions, and organic matter accumulation (Lougheed et al., 2001; Alahuhta et al., 2012; Sahuquillo et al., 2012; KISSOON et al., 2013; Wezel et al., 2013; KISSOON et al., 2015). Shallow lakes surrounded by farmlands and agriculture tend to have higher nitrogen

and phosphorus inputs than shallow lakes surrounded by forested or grassland ecosystems and therefore very different sediment chemistry. And those shallow lakes surrounded by agriculture land use tend to be more turbid than lakes surrounded by other land use types, with lower SAV species diversity (Alahuhta et al., 2012; Bayley et al., 2013; KISSOON et al., 2013; Sullivan et al., 2014; KISSOON et al., 2015). Agricultural land use is associated with increases in turbidity of shallow lakes, possibly because of increased fertilizer runoff during rain events, and the lack of plants to obstruct soil particle movement and absorb nutrients (Wezel et al., 2013). Land use seems to have an indirect effect on shallow lake plant community composition, in that land use directly affects turbidity, nutrient inputs, sediment chemistry and organic matter content, which then directly affect plant community composition and growth (Cronk and Fennessy, 2001; Loughheed et al., 2001; Alahuhta et al., 2012; Sahuquillo et al., 2012; KISSOON et al., 2013; Wezel et al., 2013; KISSOON et al., 2015).

Conclusion

Shallow lake wetlands are extremely dynamic and complex systems, ranging from clear vegetated to turbid unvegetated states (Scheffer et al., 2001; Scheffer, 2004). The stability of these states depends upon many factors including fish communities and abundance (Jeppesen et al., 1997; Zimmer et al., 2001; Norlin et al., 2006; Roozen et al., 2007; Nolby et al., 2015), nitrogen and phosphorus concentrations and inputs (Cronk and Fennessy, 2001; Bayley et al., 2007, Alahuhta et al., 2012), plant community composition (Carpenter and Lodge, 1986; Zimmer et al., 2001; Hanson et al., 2005)

Some plant species may be indicators of present shallow lake conditions, providing clues to current environmental conditions, and possibly the stability or current state (Moyle, 1945; Swartz and Gallup, 1978; Cowardin et al., 1979; Capers et al., 2009; Hansel-Welch, 2003;

Kissoon et al., 2013). Many of the studies mentioned have looked only at shallow lake plant communities in a landscape already altered by human activity. Understanding what conditions exist in a pristine setting is essential for successful restoration and conservation efforts, because studying already altered ecosystems does not provide adequate understanding of original conditions. Those studies that have assessed shallow lake ecology in an unaltered landscape have focused on phosphorus, nitrogen and water quality parameters, but have yet to describe in depth sediment chemistry and plant community species specific relationships (Bayley and Prather, 2003; Bayley et al., 2007).

Understanding the relationships within pristine clear vegetated lakes between fish communities, plant species composition, and sediment chemistry may help natural resource managers restore turbid un-vegetated lakes to their former state, or pick more appropriate restoration and mitigation sites. It is important to have excellent baseline data in order to make these management decisions and apply changes on the landscape in an effective manner from studies conducted in pristine settings. Comparing what conditions occur in pristine shallow lake settings to areas impacted by anthropogenic changes will help natural resource managers face impending changes associated with increasing human population and development and climate change.

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CHAPTER 2: MULTI-ELEMENT CHANGES IN PORE WATER OF THE EMERGENT VEGETATION ZONE OF SHALLOW LAKE WETLANDS

Introduction

Shallow lakes are unique wet ecosystems because they do not exhibit cyclical wet and dry cycles like many other types of wetlands, but are shallow aquatic systems which do not exhibit thermal stratification of the water column. Shallow lakes do exhibit alternative stable states and can switch from clear vegetated to turbid un-vegetated and back again based on environmental changes (Scheffer, 2004; Bayley, 2007; Zimmer 2009). Sediment chemistry can impact pore water and wetland ecology greatly by influencing plant communities, sediment chemistry, and turbidity (Beining and Otte, 1996; Xiaojing et al., 2006). Shallow lakes are especially vulnerable to ecological impacts from elements within pore water because of the large water/sediment interface and extensive aquatic and emergent vegetation communities. Rooted aquatic and emergent plants obtain the majority of their nutrients from the sediment and pore water located at the root zone (Cronk and Fennessy, 2001), which means pore water chemistry can greatly impact plant community composition. Many studies of shallow lake pore water have focused on the open water zone of shallow lakes and have evaluated nitrogen and phosphorus because of their importance to plant growth and recruitment (Søndergaard, 1990; Xiaojing et al., 2006; Boros et al., 2009). Less is known about the chemistry of pore water in the emergent vegetation zone in shallow lakes and how that influences the open water zone.

Amirbahman et al. (2013) found pore water phosphorus to vary seasonally, which could be linked to state changes in shallow lakes. During summer months they found reduced concentrations of phosphorus in the pore water and greater amounts of phosphorus in the water column compared to winter months. This was attributed to microbial activity and the breakdown

of organic matter on the shallow lake bottom. They also attributed these seasonal changes to reduced microbial activity in the winter months due to lower temperatures, and to faster precipitation of phosphorus out of the water column than in the summer months due to low algal growth rates. Kowalczywska-Madura and Goldyn (2012) found similar results in a deep lake, but little is known about how seasonality affects changes in concentrations of other elements within pore water, aside from nitrogen and phosphorus.

Outside influences, such as the surrounding landscape and land use practices, can greatly impact open water chemistry of shallow lakes (Zimmer et al., 2009; Kisson et al., 2015) and therefore have the potential to affect pore water chemistry in the emergent vegetation zone of shallow lakes as water moves through the emergent vegetation zone before entering the open water. A case study example of this is a wetland site in Glendalough Co, Ireland. This wetland is located within the drainage field of an abandoned mining site where zinc and lead are still seeping from the mine into the wetland (Beining and Otte, 1996). Researchers evaluated pore water chemistry and found higher concentrations of zinc, arsenic, lead, and cadmium closer to the mine than further away. Little is known how pore water chemistry changes along gradients in systems un-impacted by human influence.

Evaluating pore water within the emergent vegetation zone of shallow lake wetlands is an area still in need of research, but one which could shed light onto how quickly nutrients and runoff from the surrounding area impact the open water of shallow lakes. This study aimed to evaluate pore water chemistry in a relatively pristine setting. Other studies that have evaluated pore water chemistry have done so in impacted areas. The results of this study will lead to a better understanding of pore water chemistry in a landscape with minimal human impacts. The objectives of our study were to evaluate: 1) patterns in multi-element concentrations of pore

water along a gradient from upland areas through emergent vegetation to the open water of shallow lake wetlands, and 2) seasonal changes of multi-element concentrations of pore water during the growing season of shallow lake wetlands. Measuring pore water on the gradient from upland to open water enables us to analyze how the surrounding area is impacting the shallow lake sites. A downward gradient from upland to open water would suggest movement of an element towards the open water (the open water acting as a sink), while a reverse gradient would indicate movement away from the open water. Continuing repeated sampling over time enabled us to assess if any changes in sediment chemistry were occurring during the growing season. We expected to find differences along the gradient, because as water filters through the emergent vegetation elements and nutrients will be adsorbed to particles or absorbed by plants, with fewer chemicals or elements entering the open water of the shallow lake than coming in from the upland areas (Beining and Otte, 1996). We also expected to find differences during the season because as plants senesce nutrients will be returned to the sediment and pore water, and as temperatures vary across the season, microbial activity and its impact on nutrient and organic matter cycling would vary as well.

Methods

Study Area

Our study site was located in the northwest region of Minnesota, U.S.A, on Red Lake Nation Indian Reservation land (47.8764° N, 95.0169° W). Annual average low temperatures of -15.4 °C occur during January, while average high temperatures of 26.8 °C occur during July. Highest average annual rainfall occurs during the month of June with about 11.0 cm of precipitation, while February is the driest month with only 2.0 cm of precipitation (NOAA, 2013). Our study site, Red Lake Nation Indian Reservation, is a unique site because the land has

been under Native American jurisdiction (Meyer, 1992). It is one of the few closed reservations within the United States, meaning access by non-tribal members is restricted. As a result, the land under the jurisdiction of Red Lake Indian Reservation has experienced limited human development, with no industry or agricultural practices within its boundary (Meyer, 1992; Treuer, 2010), making it as pristine a study area as possible in this region of the United States and an ideal area to collect baseline data on conditions of un-impacted shallow lake wetlands. The wetlands chosen for this study are in the clear vegetated state and have never undergone state change since the Red Lake Department of Natural Resources began monitoring 2009-2015. The two sites (Burns Lake and Bizhiki Lake) used in this study occur south of Lower Red Lake (47°50'08.6"N, 94°53'22.9"W and 47°48'8.92"N, 95° 5'47.28"W) and are surrounded by deciduous upland forest, are not obviously connected to other wetlands or water sources, and are precipitation fed (Steward and Kantrud, 1972; Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007; Marton et al., 2015). These data will help managers to complete more successful restoration and mitigation projects, to better represent naturally occurring wetlands, and understand impacts from climate change, because this results of the study will provide an example of shallow lake ecology in their original conditions, with very low human influences.

Sampling Strategy

We chose two lakes, Burns Lake and Bizhiki Lake, which were similar to each other in size (Burns =1.39 km and Bizhiki =1.43 km circumference), plant community composition, and the characteristics of the immediate surroundings. A total of four transects at each site were established in 2014, one meter apart, starting in the transition zone from upland to wetland, and continuing to the edge of the open water, where the emergent vegetation disappeared and only submerged aquatic vegetation existed. Upland to wetland transition zones were determined by

digging a hole and observing hydric soil characteristics, soil saturation and hydric vegetation. (Mitsch and Gosselink, 2007). Along each 50 m transect, five sampling locations were positioned equidistant, at 12.5 m intervals. Positions were labeled U, indicating the start of the upland /wetland transition zone, W1, W2, and W3 indicating samples taken in the wetland emergent plant zone, and O indicating the open water of the shallow lake wetland. Dialysis vials, enabling *in situ* sampling of pore water without disturbing the pore water and surrounding area, also referred to as ‘peepers’ (Teasedale et al., 1995), were buried at each sampling location and attached to a stake by nylon fishing line for later retrieval. These dialysis vials were constructed using a 120 ml plastic sampling vial, with a hole cut in the top of the lid, and 21 μm nylon mesh filter secured between the top of the vial and the lid. Vials were filled with DI water before placement into the pore water. Starting at the end of June, 2014, the peepers were set and left for 4 weeks, then replaced with new vials. Replacement time of 4 weeks was used to ensure sampling of the pore water under normal conditions and not sampling disturbed conditions created when placing vials. This was repeated, through the end of October of that year. A spade was used to slice the sediment open to make a partition. Once the vial was placed sideways with the opening perpendicular to the surface, the sediment was pinched back together manually, to return the area as closely as possible to previous conditions. Vials were picked up and replaced in the same location each time. After a vial was retrieved, it was transported back to the lab on ice, where the water sample was filtered with a 0.45 μm Life Sciences GN-6 Metrical[®] membrane filter. Sixty ml of filtered sample was acidified with 0.01ml concentrated nitric acid, and stored for no longer than two months before multi-element analysis by Spectro Genesis SOP Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) using an Optimist nebulizer and cyclonic spray chamber. We used SmartAnalyzer Vision 4.02.034 software to run

the machine and record data with settings of plasma power set to 1425W, constant flow 13.5 L min⁻¹, auxiliary flow 1.2 L min⁻¹, nebulizer flow 0.9 L min⁻¹, and integration time of 21 seconds (see also Kisson et al., 2015). ICP-OES measures 32 different elements as follows; Ag, Al, As, B, Ba, Be, Ca, Cd, Ce, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti, Tl, V and, Zn. Detection limits for each element are listed in Table 2.1. Concentration results are a mean concentration value of 3 measurements taken per sample. We also used certified reference materials (CRM) to check machine accuracy and standardized values from different batches from CSP Science (www.cspscience.com) catalogue number 140-025-135.

Table 2.1: Detection limits determined using ICP-OES

Element	Detection Limit (mg L ⁻¹)
Ag	0.003
Al	0.007
Ar	58197.37
As	0.086
B	0.012
Ba	0.000
Be	0.000
Ca	0.017
Cd	0.003
Ce	0.040
Co	0.009
Cr	0.008
Cu	0.005
Fe	0.043
K	0.618
Li	0.001
Mg	0.084
Mn	0.001
Mo	0.016
Na	0.022
Ni	0.017
P	0.139
Pb	0.040
S	0.221
Sb	0.100
Se	0.101
Si	0.018
Sn	0.026
Sr	0.000
Ti	0.005
Tl	0.033
V	0.021
Zn	0.004

Data Analysis

Before statistical analysis element concentrations were normalized to eliminate analysis batch effects. After calculating the concentration of each element in each sample we examined the QA/QC sample results (four types in total, two different CRM, one external standard, and a batch consistency standard) of the two sampling batches. We also filtered element lines and selected those wavelengths which best matched the expected values of the CRMs. Also, eliminated elements in which greater than 50% of the samples were below the detection limit.

The statistical software R 3.2.2 was used to analyze results (R Core Team, 2015). The function `capscale` within the `vegan` package was used to analyze multivariate data. We chose multivariate data analysis techniques because the data were not normally distributed, could not be transformed to reach normal distribution, and violated assumptions of ANOVA tests. We used the function `capscale` as an unconstrained dissimilarity matrix to compare multivariate data and the Bray-Curtis distance measurement after evaluating the data set using the `rankindex` function (Oksanen et al., 2015). We performed one `capscale` analysis and overlaid 2 different sets of categorical variables on the `capscale` MDS output. Multi-element changes were compared along the gradient from upland to open water, and multi-element changes over time by categorizing these variables into sampling locations and months and tested for statistical differences of the categorical variables using the function `envfit` (Oksanen et al., 2015).

Results

Highest concentrations were observed in Ca (45.3 mg L^{-1}) and lowest concentrations were seen in Li and Zn (0.002 mg L^{-1}). Most element concentrations decreased over the sampling period with the exception of Fe (table 2.2). Only elements which resulted in concentrations above the detection limit were used in this analysis (Al, B, Ba, Ca, Fe, K, Li, Mg,

Mn, Na, P, S, Si, Sr, Zn) (table 2.2). Element concentration did not significantly change over our gradient from the edge of the upland-wetland transition zone across the emergent vegetation to the open water of the wetland. We did find month to be a significant factor with element concentrations. The capscale ordination multidimensional scaling (MDS) found the first two axes explained 53.2 % of the variation within the data set. Elements are shown in black with the element symbol indicating element location on the graphs (fig 2.1 and 2.2). We found there were no significant differences for any of the elements between sample positions along the gradient from upland to open water area ($p>0.05$). The shapes of the variables for position along the gradient do not differ greatly from one another and overlap extensively, indicating multi-element concentrations between those positions are not very different from one another. There seems to be equal variation along the x and y axes because the shapes of the position variables are stretched almost equally in the x and y direction (fig. 2.1). Elements with a 180-degree orientation from one another within the ordination exhibit a negative relationship, elements with <45-degree orientation indicate a positive relationship and elements with a 45-179-degree orientation exhibit no relationship to one another (table 2.3).

Table 2.2: Average concentrations of elements per month mg L⁻¹.

Element	Average \pm standard deviation			
	July	August	September	October
Al	0.094 \pm 0.09	0.094 \pm 0.12	0.063 \pm 0.06	0.059 \pm 0.13
B	0.010 \pm 0.01	0.008 \pm 0.005	0.010 \pm 0.005	0.010 \pm 0.004
Ba	0.048 \pm 0.05	0.041 \pm 0.04	0.042 \pm 0.03	0.037 \pm 0.03
Ca	45.367 \pm 32	36.680 \pm 24	39.625 \pm 28	36.726 \pm 22
Fe	0.576 \pm 1.30	0.489 \pm 1.28	0.403 \pm 0.89	0.092 \pm 0.18
K	1.052 \pm 0.75	0.910 \pm 0.80	1.158 \pm 1.05	1.269 \pm 0.91
Li	0.002 \pm 0.002	0.001 \pm 0.0008	0.003 \pm 0.003	0.003 \pm 0.002
Mg	14.44 \pm 9.44	11.497 \pm 7.07	10.728 \pm 6.45	10.923 \pm 6.79
Mn	0.102 \pm 0.22	0.067 \pm 0.14	0.049 \pm 0.07	0.049 \pm 0.11
Na	0.936 \pm 0.60	0.706 \pm 0.38	0.344 \pm 0.40	0.504 \pm 0.49
P	0.111 \pm 0.11	0.119 \pm 0.10	0.108 \pm 0.06	0.091 \pm 0.05
S	0.163 \pm 0.10	0.178 \pm 0.20	0.440 \pm 0.05	0.526 \pm 0.09
Si	7.519 \pm 5.27	10.518 \pm 4.68	5.310 \pm 3.29	4.467 \pm 2.75
Sr	0.038 \pm 0.02	0.031 \pm 0.02	0.032 \pm 0.02	0.032 \pm 0.02
Zn	0.002 \pm 0.001	0.004 \pm 0.01	0.004 \pm 0.01	0.004 \pm 0.002

Table 2.3: Pearson's correlation coefficients for all elements comparing average concentrations

	Al	B	Ba	Ca	Fe	K	Li	Mg	Mn	Na	P	S	Si	Sr
B	-0.74													
Ba	0.71	-0.06												
Ca	0.42	0.29	0.91											
Fe	0.82	-0.36	0.89	0.63										
K	-0.93	0.73	-0.65	-0.29	-0.89									
Li	-0.98	0.85	-0.56	-0.26	-0.72	0.91								
Mg	0.71	-0.13	0.88	0.88	0.65	-0.49	-0.61							
Mn	0.82	-0.29	0.90	0.82	0.74	-0.63	-0.73	0.98						
Na	0.87	-0.52	0.71	0.60	0.61	-0.64	-0.84	0.91	0.95					
P	0.80	-0.63	0.59	0.21	0.89	-0.96	-0.76	0.31	0.46	0.42				
S	-0.99	0.68	-0.77	-0.48	-0.89	0.95	0.95	-0.72	-0.83	-0.84	-0.84			
Si	0.89	-0.92	0.39	0.00	0.69	-0.94	-0.93	0.31	0.47	0.59	0.88	-0.87		
Sr	0.48	0.19	0.88	0.98	0.57	-0.28	-0.34	0.95	0.89	0.72	0.14	-0.51	0.03	
Zn	-0.30	-0.23	-0.63	-0.83	-0.23	0.00	0.21	-0.87	-0.78	-0.70	0.19	0.30	0.16	-0.92

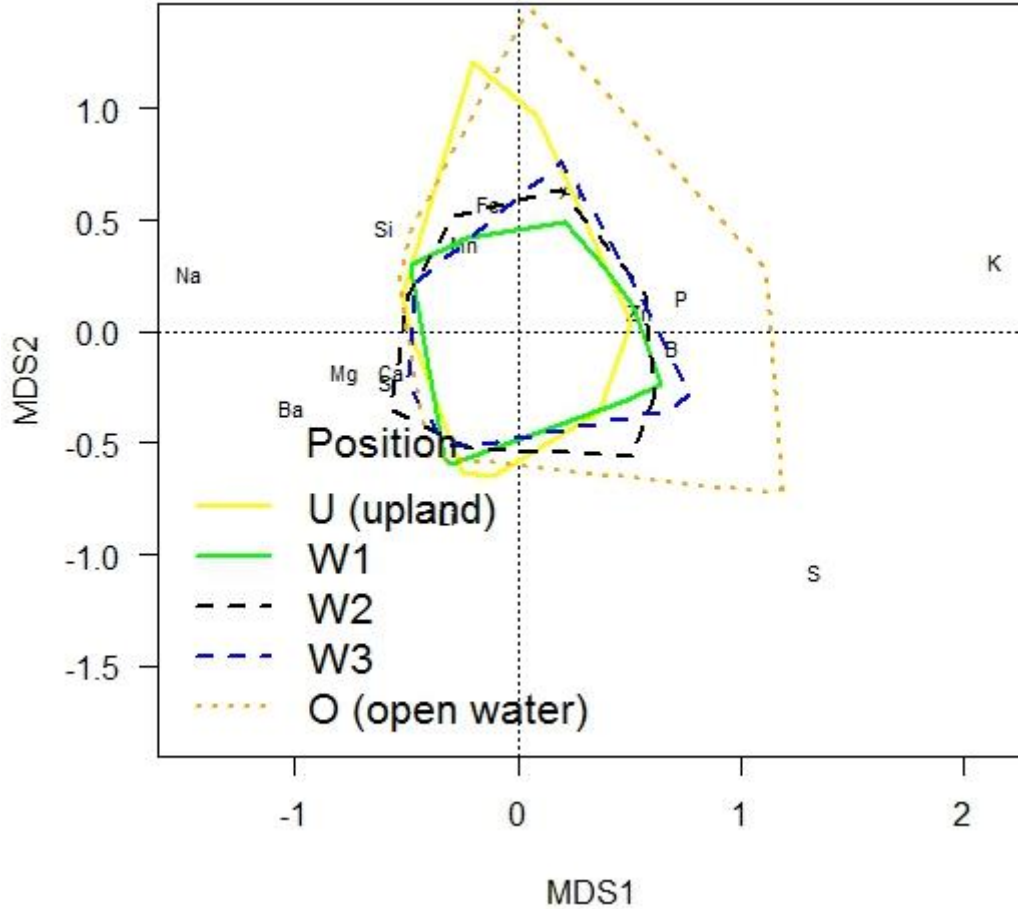


Figure 2.1: Ordination plot of the function capscale MDS in R, with elements displayed as species scores, and position variables overlaid onto the graph. Position U is the upland/wetland transition zone indicated, W1, W2, and W3 indicate positions within the emergent vegetation and position O is the open water zone. The first 2 axes explained 53.2% of the variation within the data set. Position along the gradient is not a significant factor ($p > 0.05$).

We found significant differences between months for the sampling period ($p < 0.05$). The months July and August are clustered on the left side of figure 2.2 and September and October are clustered together on the right side of the figure. There is very little variation within months on the x axis and most of the variation is seen on the y axis as the shapes are pulled more on the y axis than on the x axis (fig. 2.2).

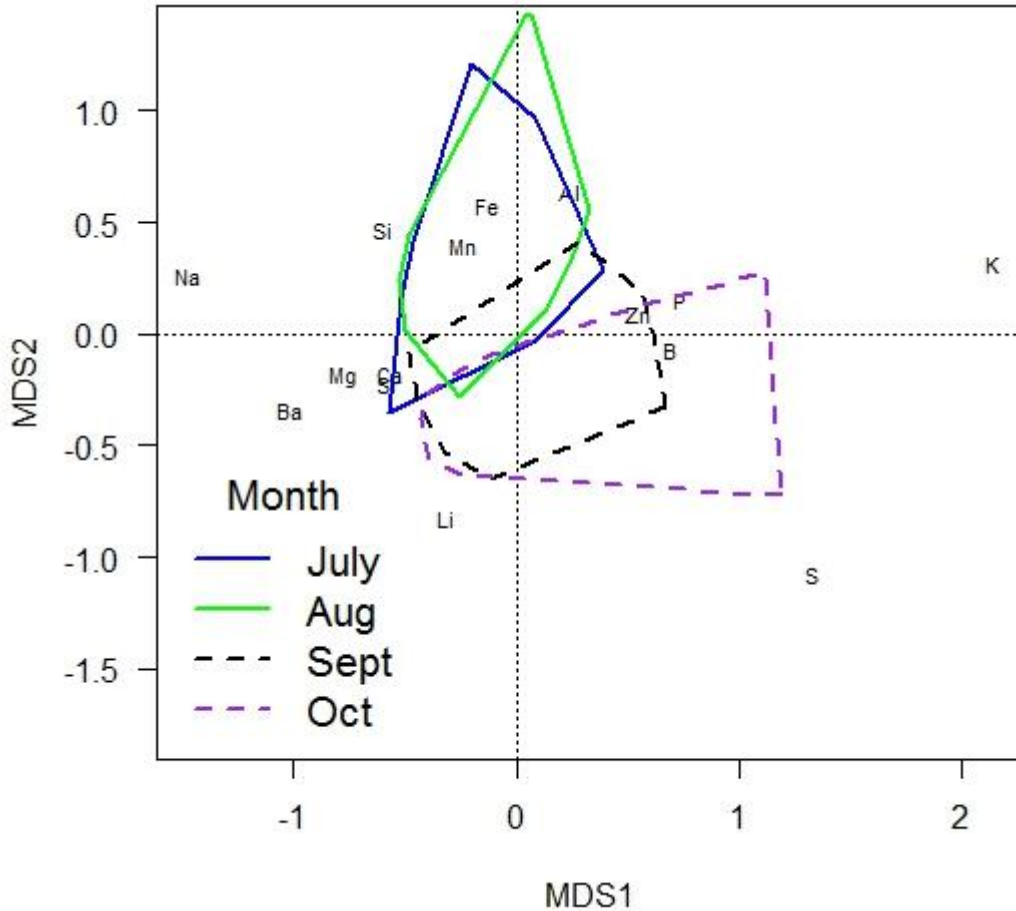


Figure 2.2: Ordination plot of the function capscale MDS in R, with elements displayed as species scores and month variables overlaid onto the graph. The first 2 axes explained 53.2% of the variation within the data set. Month is significant factor ($p < 0.05$).

Discussion

Kissoon et al. (2015) examined element concentrations within the open water and sediments of shallow lake wetlands within the Laurentian Mixed forest ecoregion of Minnesota, USA, the same ecoregion in which Red Lake Nation is located. We compared our pore water data to Kissoon et al. (2015) open water data because we did not see significant differences in the pore water and the open water in our study and because they found similar element concentrations within their open water and sediment samples. We expected to find similar element concentrations within the pore water in our study as Kissoon et al. (2015) because both studies were conducted in the same ecoregion of the same state and within a few years of one

another, but we found pore water element concentrations to be much lower than open water and sediment concentrations for all elements. The lakes in Kissoon et al. (2015) were surrounded by agriculture and human development with very little of the surrounding landscape left to forest or grasslands. This further indicates land use may greatly influence nutrient and element concentrations in wetlands, not only nitrogen and phosphorus. Other elements and nutrients, such as sulfur, are essential to plant growth, but can be toxic in high concentrations, (Cronk and Fennessy, 2001). It is therefore important when managing shallow lake wetlands to consider more than just nitrogen and phosphorus management strategies, and to manage the entirety of the ecosystem, not just the open water of shallow lake wetlands.

Compared to Bendell-Young (2002) who looked at pore water element (Ca, Mg, Mn, and Si) concentrations across nutrient gradients in peatlands north of Toronto, Canada, we found higher element concentrations of Ca, Mg and Mn in our study compared to theirs. They found pore water average Ca concentrations to range from almost 7 mg L⁻¹ in their most mineral rich sites to 0.5 mg L⁻¹ in their most mineral poor sites, Mg ranged from 0.16-1.96 mg L⁻¹, Mn ranged from .004-0.09 mg L⁻¹, and Fe ranged from 0.03-2.7 mg L⁻¹. We found Ca to range from 36-45 mg L⁻¹, Mg ranged from 10-14 mg L⁻¹, Mn ranged from 0.04-0.10 mg L⁻¹, and Fe ranged from 0.09-0.5 mg L⁻¹. Bendell-Young (2002) found that in their most mineral poor sites the sources of Ca, Mg, Mn, and Fe were from precipitation while in the mineral rich sites sources were from weathering of parent rock material and ground water inputs. This may indicate that mineral inputs in our sites are due to ground water inputs and not precipitation. If our sites are already receiving nutrient and mineral inputs from ground water, receiving even more inputs from outside sources such as runoff could change clear lakes to turbid lakes. Understanding where element and nutrient concentrations are derived from is important in understanding shallow lake

ecology and state stability. Additional nutrient inputs from runoff and industrial pollution could cause algal blooms in the open water of shallow lakes. If ground water inputs already contain high element and nutrient concentrations, accumulation over time could induce a change in state from clear to turbid without additional inputs from runoff and pollution sources.

SurrIDGE et al. (2005) examined phosphorus concentrations in pore water of Strumpshaw Fen in the United Kingdom. In their study they found P concentrations as high as 4.5 mg L^{-1} whereas our study had concentrations up to 0.111 mg L^{-1} . The landscape surrounding Strumpshaw Fen is very different than the landscape surrounding Red Lake Nation, and the two lakes used in our study. Strumpshaw Fen is surrounded immediately by heavy agriculture and urbanization, whereas the lakes used in our study are surrounded by upland forest with the nearest agricultural lands greater than four km away. The differences in land use may be what is driving the large difference between phosphorus in the pore water between our study and SurrIDGE et al.'s (2005), indicating that agriculture land use practices can be sources of phosphorus deposition within the pore water of wetland ecosystems. SurrIDGE et al. (2005) also indicated that nutrients within pore water of wetlands can act as sources during flooding and rain events and can transfer nutrients into connected water bodies. The pore water in our study site is then not contributing high levels of phosphorus to the open water zone of the shallow lake wetlands in our study site, which could be an explanation for why our sites have remained in the clear state.

The emergent vegetation area of shallow lake wetlands may act as a source of nutrients and elements during high water events (SurrIDGE et al., 2005), but in our study we were unable to see changes in element concentration across the gradient from the surrounding landscape into the open water zone. We may not have seen significant differences along the gradient from upland

to the open water of the wetland because the emergent vegetation along that zone had created a floating mat of vegetation. There was still quite a bit of water movement through and under this floating mat which may have provided extensive mixing of the pore water just as wave action mixes the open water of shallow lake wetlands (Jackson, 2003; Scheffer, 2004). The length of the gradient was also small, spanning 50 m, which could also attribute to why we did not see significant variation in element concentrations along the gradient. In studies where element concentrations have changed along a gradient the gradient was much longer, and had significant human impacts at a specific source site (Beining and Otte, 1996; Novak and Pacherov, 2007). If there was a longer emergent vegetation gradient between the edge of the upland and the open water of shallow lake wetlands, we may expect to see more of a gradient with element concentrations, because they would move slower through the gradient from the upland zone to the open water. Novak and Pacherov (2007) found metal concentrations in surface pore water of bog sites closer to industries to be significantly higher than those further away, but found no significant differences between the two sites in pore water concentrations 40 cm below the sediment surface. Element concentrations at the 40 cm depth were similar to those surface concentrations of the sites closer to pollution sources. This indicates mobility of metals and associated ions within the vertical profile of the sediment as well as mobility through hydrologic movements from point source pollution. We may not have seen significant changes across the gradient in our study because perhaps there was significant vertical movement of elements in the sediment profile.

One explanation for month being a significant factor in this study could be plant senescence. Plant senescence started in August and by the end of September, when the dialysis vials were collected and replaced for the 3rd time, senescence of most plant species had largely

already taken place. As plants senesce, they decompose and return nutrients back to the ecosystem as the organic matter decays and breaks down (Frazier et al., 1996; Cronk & Fennessy, 2001). This could be an explanation for why we saw an increase in some element concentrations after August, because as plant matter was breaking down, elements which had been bound up in plant matter were then released into the pore water. Decreases in temperature from June to October could also contribute to element concentration changes as microbe activity slows down (Devito and Hill, 1998; Amirbahman et al., 2013). We may have seen a decrease in some elements because, as plant roots stop oxidizing the pore water at the root zone, some elements may have been chemically reduced, making them less water soluble and therefore not seen in the pore water (Reddy & DeLaune, 2008).

Conclusions

The data generated from this study can be used as reference data for wetland restoration and conservation efforts. Little is known about pore water chemistry in un-impacted shallow lake wetlands, and this study adds to a better understanding of shallow lake wetland ecology. It is also important to note that pore water chemistry changes are seasonal, and may be due to plant senescence. This is important to realize because many wetland water quality monitoring activities take place on a yearly basis. Potential impacts of climate change may be changes in the timing of plant senescence. If monitoring activities keep taking place at the same time each year, without regarding plant senescence, managers and biologists could see changes in water chemistry that do not accurately reflect previous conditions. For example, if measurements are taken first week of September each year after plant senescence, but with climate change plant senescence in future years is not starting until the 3rd week of September, the water quality may change, but the conditions you are measuring have also changed.

It is also important to note changes in element concentrations of pore water in wetlands for restoration or mitigation planting purposes. Micronutrients are important for plant growth and development (Cronk & Fennessy, 2001), so understanding when those micronutrients and elements are most available is important so the desired plant species establish and have the best chance at survival. Also understanding if element and nutrient concentrations are derived from precipitation or ground water inputs may help manage state stability. Wetland restoration and mitigation is expensive and time consuming, and these results may help with better planning and establishment of wetland plants in restoration projects. This study gives a better understanding of what elements are present in pore water with very little surrounding landscape use. These results then will help with restoration because examining pore water at a restoration site and comparing those values to our study will help evaluate what elements are being contributed from the surrounding landscape. Appropriate plant species and structure of the restoration then can then be chosen to achieve the goals and objectives of the restoration.

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CHAPTER 3: UNDERSTANDING THE RELATIONSHIPS BETWEEN SHALLOW LAKE WETLAND FISH COMMUNITIES AND SUBMERGED AQUATIC VEGETATION

Introduction

Shallow lake wetlands are unique aquatic systems because they are typically dominated by densely colonized macrophytes, and do not experience the thermal stratification of deeper lakes (Scheffer, 2004). This means shallow lakes do not have the same characteristics of deep lakes in terms of fish and plant communities, nutrient exchange, light availability, and physical factors. The term shallow lake refers to a waterbody less than three meters deep (not restricted in surface area size) and surrounded by emergent wetland plant species (Scheffer, 2004). Shallow lakes function differently than deep lakes which exhibit thermal stratification and wetlands which undergo wet and dry cycles because of extensive water sediment interactions and high macrophyte colonization (Scheffer, 2004; Wezel et al., 2013).

Shallow lake wetlands are also unique in that they can exhibit alternative stable states, changing from a clear vegetated state to a turbid non-vegetated state. Research has yet to uncover the thresholds and causes of these shifts (Scheffer et al., 2001; Bayley and Prather, 2003; Scheffer, 2004; Van Geest et al., 2005; Bayley et al., 2007; Zimmer et al., 2009; Hobbs et al., 2012). Jackson (2003) revealed that the state of a shallow lake is probably caused by interactions within each lake and the immediate surrounding area, and is not an effect of region or latitude. This is because shallow lakes within the same region exhibit both the clear and the turbid state, even when they are impacted by the same climatic effects and land use practices. Benthivorous fish can uproot and kill submerged aquatic vegetation (SAV), stir up sediment, and decrease water clarity as they feed, leading to a shift from a clear vegetated state to a turbid un-

vegetated state (Zimmer et al., 2003; Scheffer, 2004; Roozen et al., 2007). The presence of fish communities has been linked to lower abundance of SAV, increased phytoplankton, and increased turbidity in shallow lakes (Hanson and Butler, 1994b; Zimmer et al., 2001; Zimmer et al., 2002; Hanson et al., 2005). Jeppesen et al. (1999) found turbid lakes, compared with clear lakes, had higher fish biomass, which was then associated with higher chlorophyll *a*, turbidity, and decreased macrophyte abundance. The turbid lakes were dominated by planktivorous fish species, while the clear lakes had greater piscivorous fish abundance, indicating fish community composition and food web interactions play a role in the state stability of shallow lakes. Fish community composition also impacts shallow lake state stability, not solely the presence or absence of fish. Those lakes dominated by benthivorous fish species are more likely to be turbid, because as these fish feed, they re-suspend sediment particles into the water column, making essential nutrients more available to phytoplankton. This results in increased phytoplankton abundance and therefore turbidity (Zimmer et al., 2001; Roozen et al., 2007).

Although many studies have documented food web interactions with fish, zooplankton, and SAV, few studies have done so in a pristine setting (Jackson, 2003; Bayley et al., 2007). It is poorly understood if species specific relationships between fish and SAV occur in the shallow lakes, and if this contributes to state stability changes. Understanding fish communities in pristine wetlands is important because it may lead to a better understanding of how these ecosystems function with little to no anthropogenic influences, and how those fish communities influence other ecological characteristics. This in turn will help with management decision making, and better restoration and conservation efforts. By characterizing pristine shallow lakes, we will be able to identify functional redundancy of species and where niche partitioning occurs. The objective of our study was to evaluate the influence of fish communities on submerged

aquatic vegetation communities in order to broaden the understanding of the roles of planktivorous and benthivorous fish, and to increase available information relating to alternative stable states in a pristine setting. We expected to see similar, but opposite, changes in mass of fish and SAV, and significantly higher SAV mass in fishless lakes compared to lakes with fish if fish were negatively impacting plant communities.

Methods

Study Area

Our study site was located in the northwest region of Minnesota, U.S.A, on Red Lake Nation Indian Reservation land (47.8764° N, 95.0169° W). Annual average low temperatures of -15.4 °C occur during January, while average high temperatures of 26.8 °C occur during July. Highest average annual rainfall occurs during the month of June with about 11.0 cm of precipitation, while February is the driest month with only 2.0 cm of precipitation (NOAA, 2013). Our study site, Red Lake Nation Indian Reservation, is a unique site because the land has always been under Native American jurisdiction. It is one of the few closed reservations within the United States, meaning access by non-tribal members is restricted. As a result, the land under the jurisdiction of Red Lake Indian Reservation has experienced limited human development, with no industry or agricultural practices within its boundary (Meyer, 1992; Treuer, 2010), making it as pristine a study area as possible in this region of the United States and an ideal area in which to collect baseline data on of shallow lake wetlands with few anthropogenic influences. The wetlands chosen for this study are in the clear vegetated state ($\text{Chl } a < 18 \mu\text{g L}^{-1}$, Bayley et al., 2009) and have never undergone state change during the monitoring period by Red Lake Department of Natural Resources from 2009-2015. These data will help managers to complete

more successful restoration and mitigation projects which will better represent naturally occurring wetlands.

Sampling Strategy

We assessed the fish communities in 24 shallow lake wetland sites. We sampled sites in two different landscape locations, with 12 lakes located in a bog landscape on the west side of Lower Red Lake (48° 0'5.41"N 95°26'0.82"W) and 12 lakes that were considered isolated wetlands as defined by Leibowitz and Nadeau (2003), located south of Lower Red Lake (47°48'8.92"N, 95° 5'47.28"W). To sample the fish community within each lake we used three fyke nets, and one experimental gill net, all of which were set for a single 24-hr period. Randomized sampling took place from late June to early August of 2009, 2010, 2011, 2014, and 2015. The 3 fyke nets (6.5 mm bar mesh with 4 hoops, 1 throat, 7.62 mm lead and a 0.69 m X 0.99 m rectangular frame opening into the trap) were set equidistant to one another throughout the shallow lake wetlands, and set perpendicular to shore. This allows the lead line to direct fish into the fyke net. One experimental gill net (61.0m multifilament net with 19, 25, 32, 38, and 51-mm bar meshes) was set in the middle of each shallow lake. Both types of nets were set overnight, for a period not to exceed 24 hours. The use of the two types of nets allows for the collection of the entire fish community. Upon retrieval, fish were sorted by species, and weighed. We expected to find populations of bullhead and minnows such as *Ameiurus melas* (black bull head), *Ameiurus nebulosus* (brown bullhead), *Umbra limi* (central mud minnows), *Pimephales promelas* (fathead minnows), *Phoxinus neogaeus* (finescale dace), and *Phoxinus eos* (northern red belly dace).

SAV community composition was sampled using a rake throw technique. A common garden rake with a dual sided head was thrown out of both sides of the canoe, dredged along the

bottom, and pulled up. The vegetation collected on the rake head was weighed and the percentage of each species of plant was estimated. Sampling took place along 4 transects with 5 sampling stopping points spaced equidistant along each transect for a total of 20 sampling points, and 40 rake throws per lake. Sampling occurred at the same time fish sampling took place, in the same 24 shallow lake wetland sites, from late June to early August in 2009, 2010, 2011, 2014 and 2015.

Water quality parameters were measured taking a single dip sample in all 24 shallow lake sites, in the middle of the lake. We used multi-probe models YSI 6920 V2-2 and YSI 6920 V2-2 to measure water temperature, conductivity, pH, and dissolved oxygen. We also measured turbidity in-house using USEPA method 180.1 with a HACH 2100Q turbidity meter. Alkalinity was measured in-house using HACH method 8221. The following were the parameters analyzed by RMB Environmental Laboratories in Detroit Lakes, MN followed by their EPA method number; Total phosphorus (TP): EPA 365.3, Orthophosphate (OP): EPA 365.3, Total Dissolved Phosphorus (TDP): EPA 365.3, Inorganic nitrogen (nitrate & nitrite nitrogen) (listed as N+N or N2N3): EPA353.2 Rev 2.0, Total Kjeldahl Nitrogen (TKN): EPA 351.2 Rev 2.0, Ammonia-nitrogen (NH3): EPA 350.1 Rev 2.0, Sulfate (SO4): ASTM D516-07, Dissolved Organic Carbon (DOC): SM5310 C-96.

Statistical Techniques

We characterized fish and SAV communities by calculating the average biomass per species per year of the study. Average species richness was determined for each year of the study and for the two geographic locations (bog and isolated sites). We tested for significant changes in species richness of SAV and fish using a t-test to compare 2009 to 2015 and bog to isolated sites each year of the study (data were not normally distributed and could not be

transformed to normal distribution so an ANOVA test could not be used for comparison). We also used t-test comparisons to determine if water quality parameters differed significantly between the bog and isolated sites.

We evaluated the impact fish communities had on SAV communities by first comparing total fish biomass to total plant biomass in all of our 24 sites, and also by location. We compared biomass data from 2009 to 2015 using a t-test to see if significant changes in mass occurred for both plant biomass and fish biomass for all sites, for bogs sites, and for isolated sites. We also used a t-test to compare SAV biomass in lakes with fish to fishless lakes in all years. We used multivariate statistical techniques to assess further if there were relationships between fish and SAV communities using non-metric multidimensional scaling (NMS) in PC-ORD 6 (McCune and Mefford, 2001) as described by Kruskal (1964) and Mather (1976), using the Sørenson distance measure, with the “slow and thorough” procedure, with a random starting position and 250 runs with real and randomized data. NMS was chosen as the statistical technique because it is recommended for finding relationships in multivariate ecological data and able to assess non-linear data sets with different scales of measurement (McCune and Grace, 2002). Prior to NMS SAV biomass data were 0.25 power transformed to relativize the data in order to account for differences in magnitude of species sample masses (McCune and Grace, 2002). We assessed species specific relationships using Pearson and Kendall correlations with Ordination axis to see if specific fish and SAV correlated with the axes used. We then applied Multi-response permutation procedures (MRPP), using the Sørenson distance measure, to test for significance of our categorical variables; year, location, and fish type (Mielke and Berry, 2001).

Results

We found a significant decreasing trend in average fish biomass from 2009 to 2015 with average biomass ranging from 7.7 ± 4.0 kg in 2009 to 1.3 ± 0.6 kg in 2015 ($p < 0.05$) (fig. 3.1).

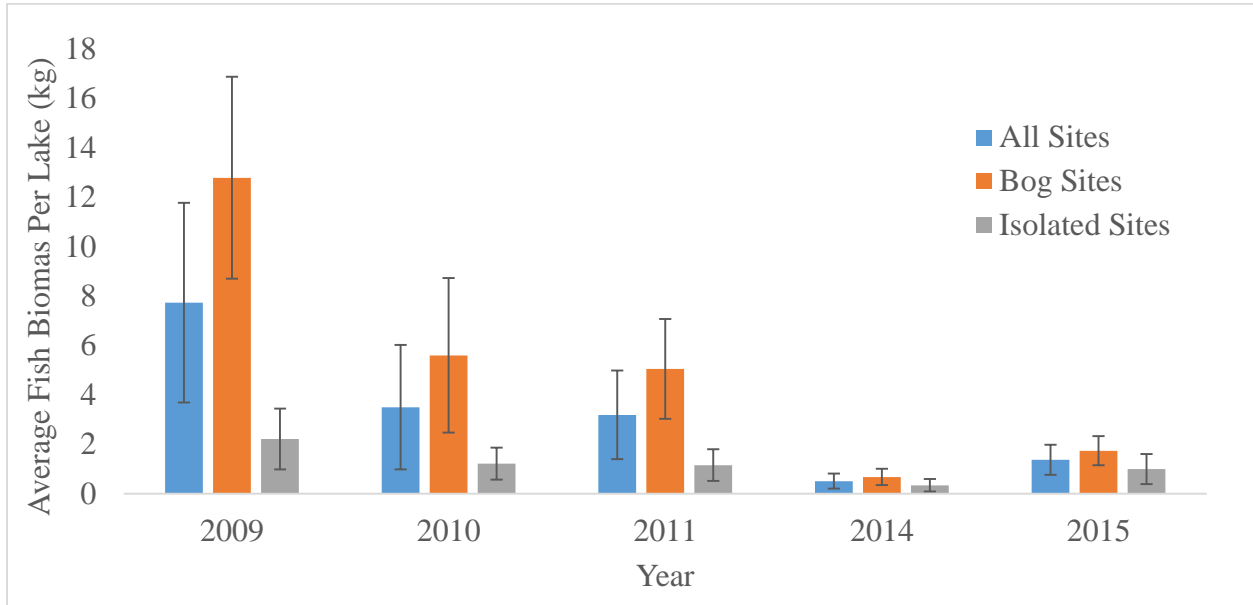


Figure 3.1: Average fish biomass (\pm SD) from 2009 to 2015. No data were collected in 2012 and 2013. There was significantly ($p < 0.05$) greater biomass in 2009 than in 2015 when comparing all sites. Bog sites had significantly greater biomass than isolated sites in 2009, 2010, and 2011. No data was collected in 2012 and 2013.

Furthermore, the 12 bog sites had significantly higher fish biomass than the isolated sites in 2009, 2010 and 2011 ($p < 0.05$), but not in 2014 and 2015 ($p > 0.05$). Fish biomass of bog sites ($n=12$) ranged from 12.8 ± 2.0 kg in 2009 to 1.7 ± 0.23 kg in 2015 and at isolated sites ($n=12$) from 2.2 ± 1.2 kg in 2009 to 1.0 ± 0.6 kg in 2015. Fish species richness did not significantly change from 2009 to 2015 ($p > 0.05$) with 4.0 ± 1.1 species in 2009 to 3.6 ± 1.1 species in 2015 (fig. 3.2). Bog sites had significantly higher fish species richness in 2009, 2010 and 2011 than the isolated sites.

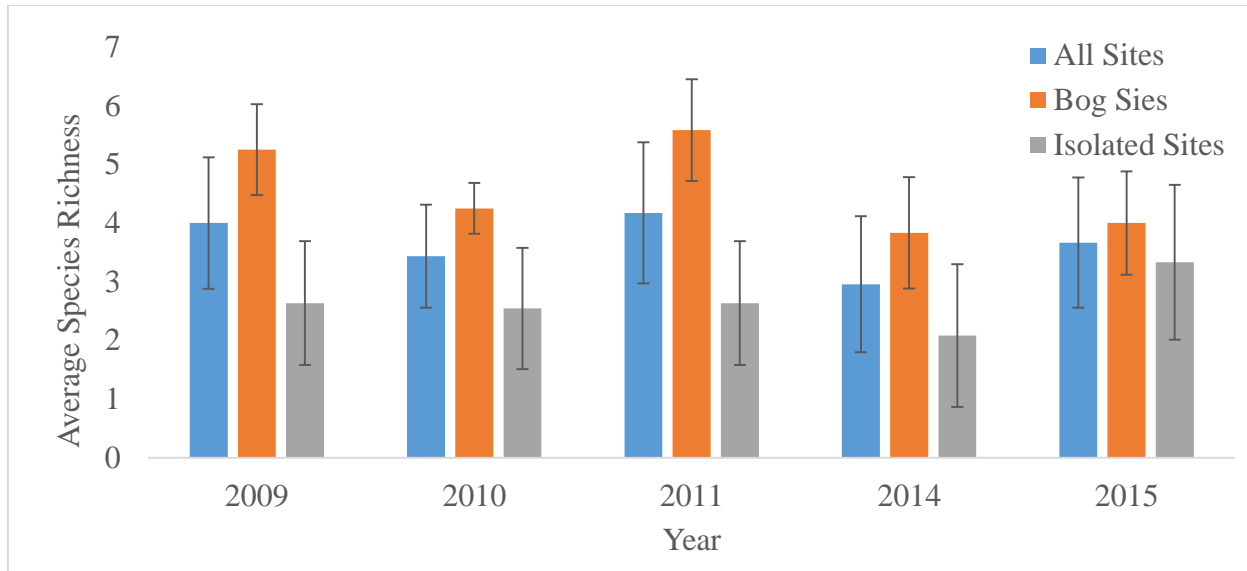


Figure 3.2: Average fish species richness (\pm SD) for each year. No significant difference in all sites between 2009 to 2015, but in 2009, 2010 and 2011 the bog sites had significantly greater species richness than the isolated sites. No data were collected in 2012 and 2013.

There were no fish species that only occurred in the isolated sites. A species occurred either in both locations or only in the bog sites. We had four sites that did not contain any fish. One of the fishless sites was located in the bog region, the other three were located in the isolated sites. Species such as *Pimephales promelas* (fathead minnow), *Chrosomus eos* (northern redbelly dace), and *Umbra limi* (central mud minnow) were the only species seen in both locations and all years of the study. *Esox lucius* (northern pike) were found only in the bog sites in all years of the study (fig. 3.3). *Noturus gyrinus* (Madtom tadpoles) were also only found in the bog sites but only in years 2009, 2011 and 2015, while *Perca flavescens* (yellow perch) and *Notemigonus crysoleucas* (golden shiners) were found only in bog sites during 2009 and 2010 (table 1). Species with the highest average biomass in the bog sites were *Ameiurus melas* (black bullhead), *Pimephales promelas* (fathead minnow), *Chrosomus eos* (northern redbelly dace), and *Perca flavescens* (yellow perch), while species with the lowest average biomass included

Lepomis macrochirus (bluegill), *Luxilus cornutus* (common shiner), *Noturus gyrinus* (tadpole madtom) and *Notropis heterolepis* (blacknose shiner). Species with the highest average biomass in the isolated sites were *Pimephales promelas* (fathead minnow) and *Chrosomus eos* (northern redbelly dace), and species with the lowest average biomass included *Ameiurus melas* (black bullhead) and *Lepomis macrochirus* (bluegill) (fig. 3.4).

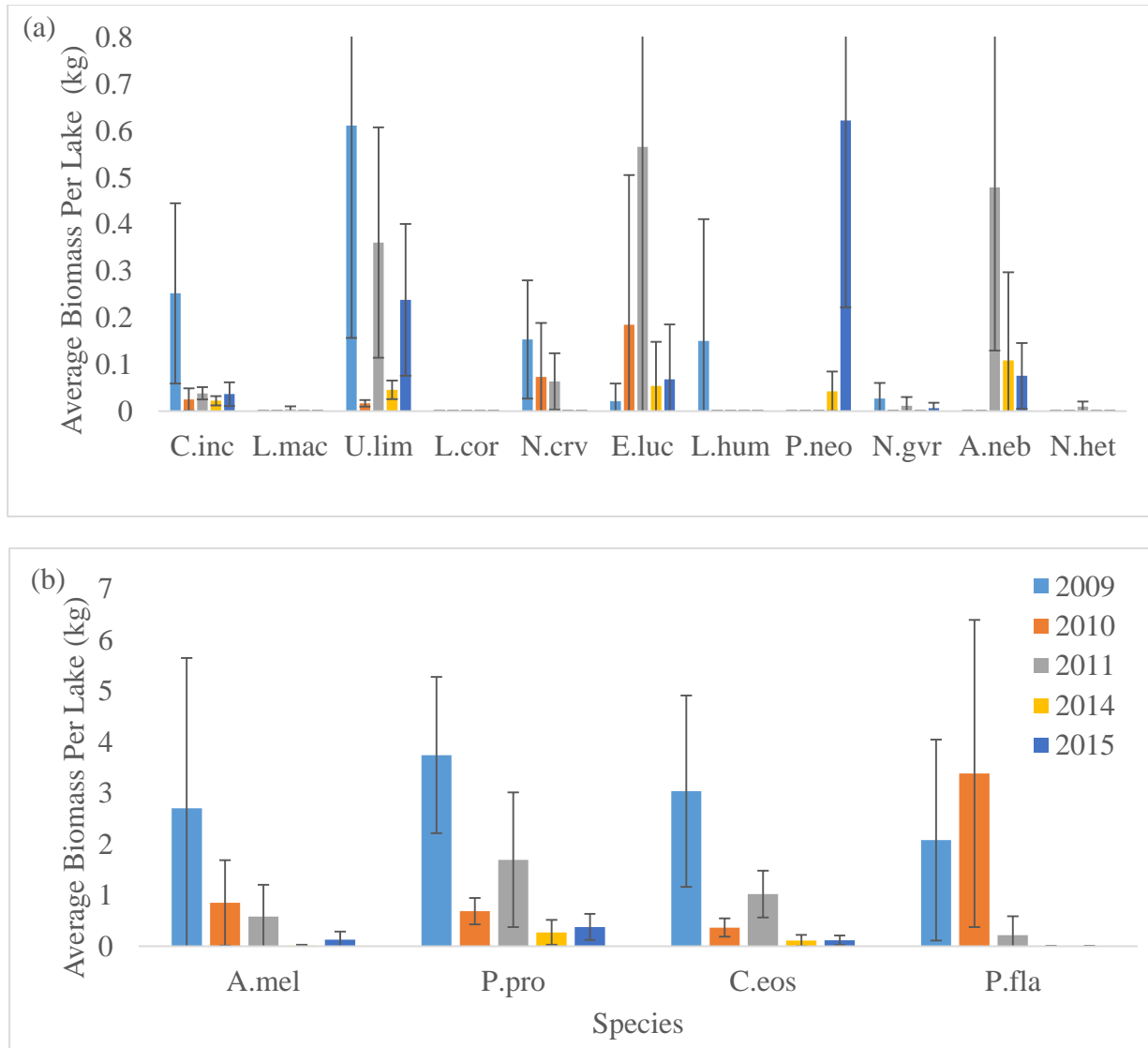


Figure 3.3: Average fish biomass (\pm SD) for bog sites per species over each year of the study period, (a) y-axis scale ranges from 0-0.8 kg and (b) y-axis scale ranges from 0-7 kg. Species names are abbreviated with the first letter of the genus name followed by the first 3 letters of the species name. Full names can be found on Table 1.

Table 3.1: Presence of each fish species is indicated by an X and absence is indicated by a 0 for the bog and isolated site locations for 2009-2015. No data were collected in 2012 and 2013.

Scientific Name	Common Name	2009		2010		2011		2014		2015	
		Bog	Isolated	Bog	Isolated	Bog	Isolated	Bog	Isolated	Bog	Isolated
<i>Notropis heterolepis</i>	Blacknose shiner	0	0	0	0	X	0	0	0	0	0
<i>Ameiurus melas</i>	Black Bullhead	X	X	X	0	X	X	X	0	X	0
<i>Ameiurus nebulosus</i>	Brown Bullhead	0	0	0	0	X	0	X	0	X	0
<i>Lepomis macrochirus</i>	Bluegill	0	0	0	X	X	0	0	0	0	0
<i>Culaea inconstans</i>	Brook stickleback	X	X	X	X	X	X	X	X	X	X
<i>Umbra limi</i>	Central mudminnow	X	X	X	X	X	X	X	X	X	X
<i>Luxilus cornutus</i>	Common shiner	0	0	0	0	0	0	X	0	0	0
<i>Pimephales promelas</i>	Fathead minnow	X	X	X	X	X	X	X	X	X	X
<i>Phoxinus neogaeus</i>	Finescale dace	0	0	0	0	0	0	X	X	X	X
<i>Notemigonus crysoleucas</i>	Golden Shiner	X	0	X	0	X	0	0	0	0	0
<i>Chrosomus eos</i>	Northern redbelly dace	X	X	X	X	X	X	X	X	X	X
<i>Esox lucius</i>	Northern pike	X	0	X	0	X	0	X	0	X	0
<i>Lepomis humilis</i>	Orange spotted sunfish	X	0	0	0	0	0	0	0	0	0
<i>Noturus gyrinus</i>	Tadpole madtom	X	0	X	0	X	0	0	0	X	0
<i>Perca flavescens</i>	Yellow perch	X	0	X	0	X	0	0	0	0	0

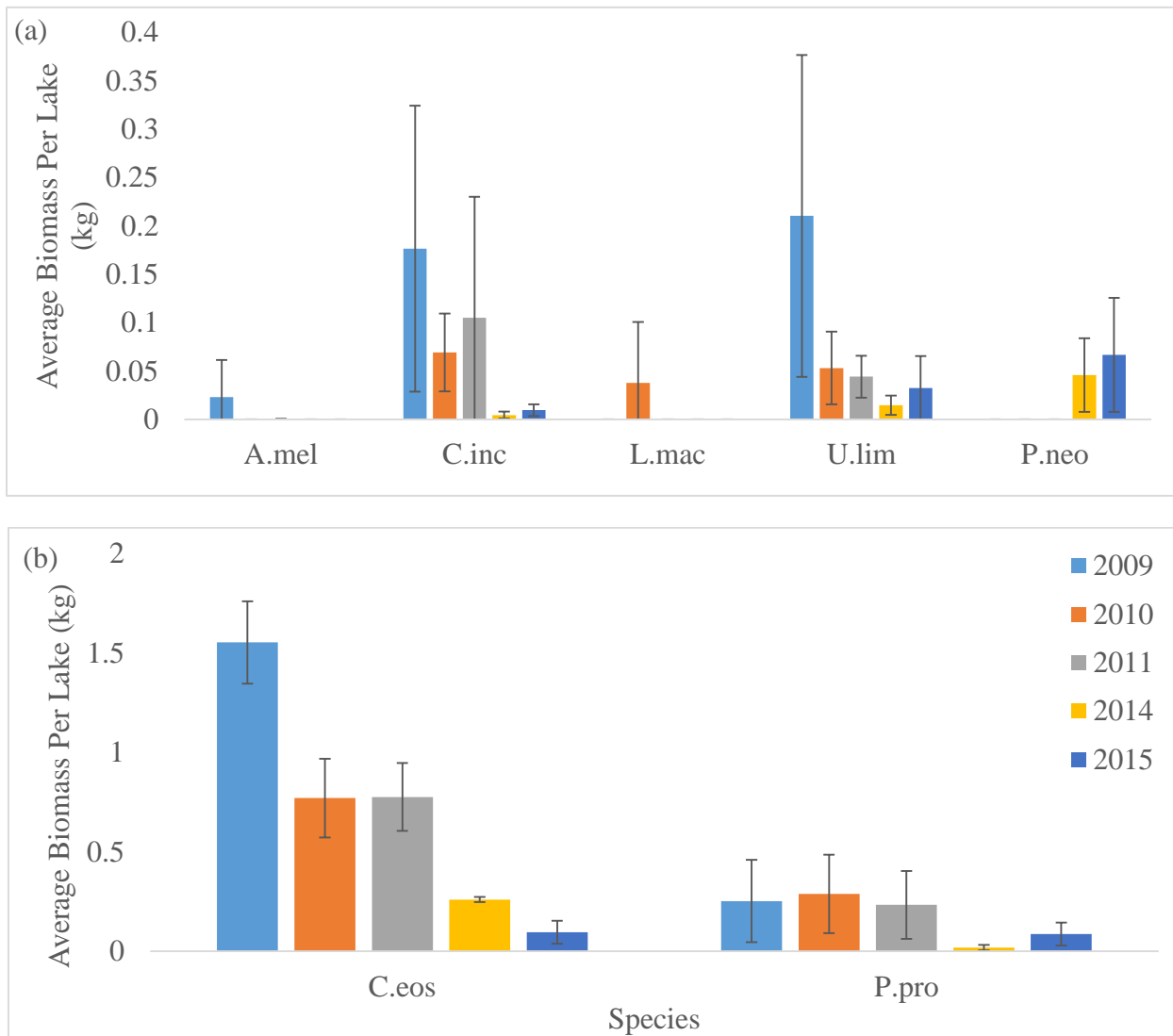


Figure 3.4: Average fish biomass (\pm SD) for isolated sites per species over each year of the study period, (a) y-axis ranges from 0-0.4 kg, (b) y-axis ranges from 0-2 kg. Species names are abbreviated with the first letter of the genus name followed by the first 3 letters of the species name. Full names can be found in Table 3.1.

We did not find a significant trend in SAV biomass data over the period of the study ($p > 0.05$). Highest average SAV biomass occurred in 2010 (0.47 ± 0.22 kg) and lowest average SAV occurred in 2015 (0.30 ± 0.11 kg). We found no significant differences in average SAV biomass between fishless lakes ($n=4$) and those with fish ($n=20$), with average SAV biomass ranging

from 0.20 – 0.43 kg in sites with fish and 0.29-0.49 kg in fishless sites. We found isolated sites had significantly higher biomass than bog sites in 2010 and 2014 ($p < 0.05$), but not in 2009, 2010, or 2015 indicating no strong pattern of SAV biomass difference between the two locations. Average SAV biomass ranged from 0.26-0.31 kg in the bog sites and 0.33-0.67 kg in the isolated sites (fig. 3.5). Average SAV species richness significantly increased from 2009 to 2015 ($p < 0.05$) when comparing average species richness through all sites.

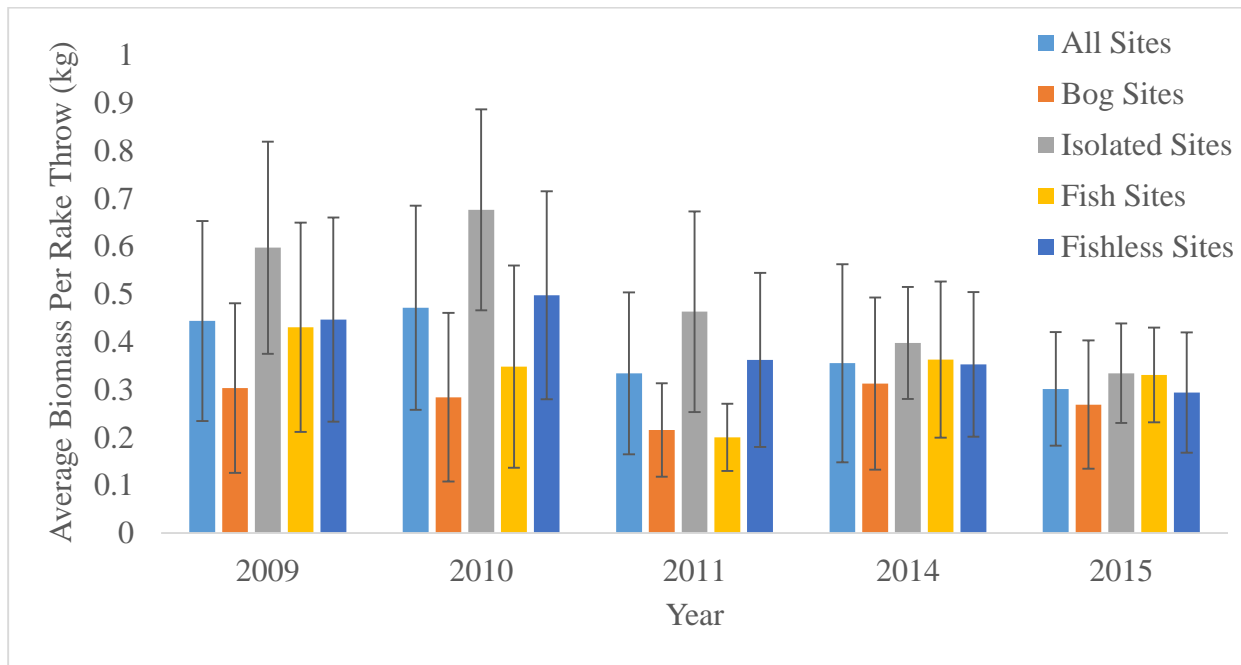


Figure 3.5: Average submerged aquatic vegetation (SAV) biomass (\pm SD) per year. There was no significant difference in the average biomass in all sites from 2009 to 2015. There was no significant difference in any year between fishless sites and sites with fish. When comparing bog sites to isolated sites, isolated sites had significantly higher average SAV in 2010 and 2015.

There were no significant differences in SAV species richness in any year between the bog and isolated sites ($p > 0.05$), but there was significantly greater species richness in sites with fish than fishless sites in 2010 only (fig. 3.6). When comparing species presence based on location, *Carex* spp. and *Lemna trisulca* were observed only in isolated sites and not found in

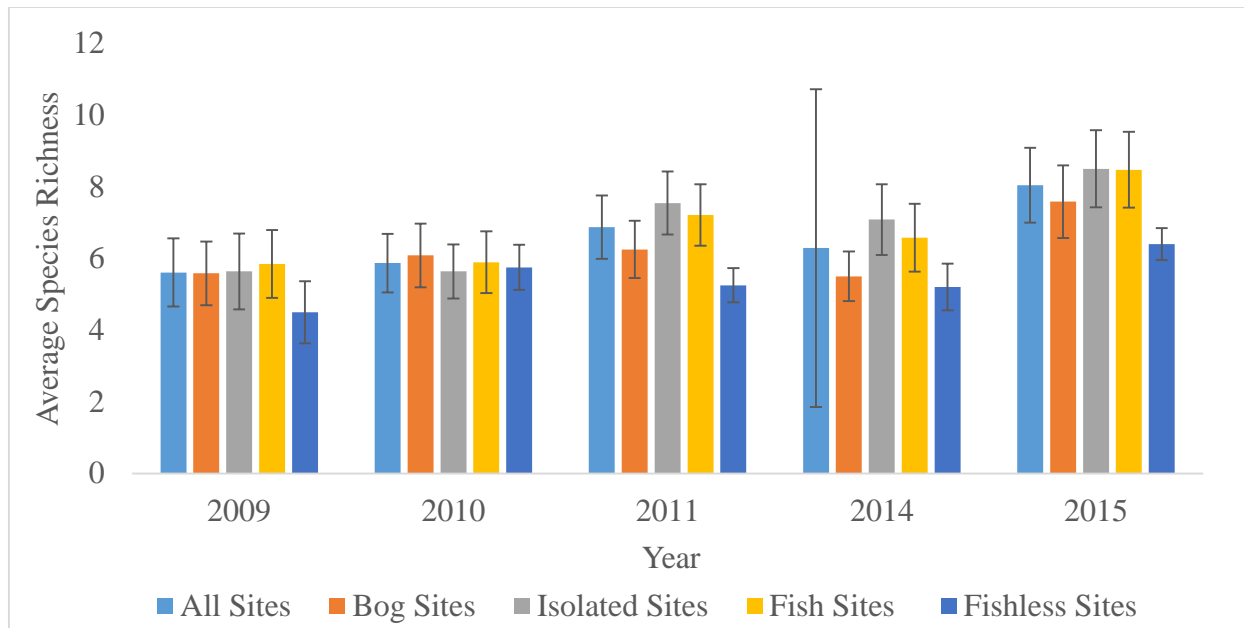


Figure 3.6: Average submerged aquatic vegetation (SAV) species richness (\pm SD) per year. Significantly greater species richness was seen in 2015 compared to 2009 when looking at all 24 sites. There were no significant differences in species richness between bog and isolated sites for any year. When comparing fishless sites and those with fish, sites with fish had significantly greater species richness in all years except 2010.

bog sites. All other species were found in the bog and isolated sites. When comparing plant species presence based on fish presence or absence all species occurred in sites with fish, while *Carex* spp., filamentous algae, *Potamogeton gramineus*, and *Potamogeton robbinsii* did not occur in fishless sites. *Chara* spp., *Myriophyllum sibiricum*, *Najas flexilis*, *Nuphar microphylla*, *Potamogeton amplifolius*, *Potamogeton natans*, and *Vallisneria americana* were found in all years in all sites regardless of location or fish presence (table 3.2). The bog sites were dominated by *Chara* spp., *Najas flexilis*, and *Myriophyllum sibiricum* (fig. 3.7). Dominant species for the isolated sites include *Chara* spp. and *Najas flexilis* (fig. 3.8). Sites with fish were dominated again by *Chara* spp. and *Najas flexilis* while sites without fish were dominated by *Chara* spp., *Najas flexilis*, *Myriophyllum sibiricum* and *Potamogeton zosteriformis* (fig. 3.9 and fig. 3.10).

Table 3.2: Presence of each SAV species is indicated by an X and absence is indicated by a 0 for 2009-2015 (No data were collected in 2012 and 2013). Column headings under each year are abbreviated as follows; B = bog sites, I = isolated sites, F = sites with fish, Fø = fishless sites.

Scientific Name	2009				2010				2011				2014				2015			
	B	I	F	Fø	B	I	F	Fø	B	I	F	Fø	B	I	F	Fø	B	I	F	Fø
<i>Brasenia schreberi</i>	X	X	X	X	X	X	X	0	X	X	X	0	0	0	0	0	X	X	X	X
<i>Carex</i> spp.	0	X	X	0	0	0	0	0	0	X	X	0	0	0	0	0	0	0	0	0
<i>Ceratophyllum demersum</i>	X	X	X	0	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X
<i>Chara</i> spp.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Filamentous algae</i>	0	0	0	0	0	0	0	0	X	0	X	0	0	0	0	0	X	X	X	0
<i>Lemna trisulca</i>	0	X	0	X	0	X	0	X	0	X	0	X	0	0	0	0	0	X	X	0
<i>Myriophyllum sibiricum</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Najas flexilis</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Nuphar microphylla</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Nymphaea alba</i>	0	X	X	X	0	X	X	X	0	X	X	X	X	X	X	X	0	X	X	X
<i>Potamogeton amplifolius</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Potamogeton friesii</i>	0	0	0	0	0	0	0	0	0	0	0	0	X	X	X	X	X	X	X	0
<i>Potamogeton foliosus</i>	0	0	0	0	0	X	X	0	0	X	X	0	0	X	0	X	X	X	X	0
<i>Potamogeton gramineus</i>	X	X	X	0	0	0	0	0	X	X	X	0	0	0	0	0	0	X	X	0
<i>Potamogeton natans</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Potamogeton praelongus</i>	0	0	0	0	0	0	0	0	0	0	0	0	X	X	X	X	X	X	X	X
<i>Potamogeton pusillus</i>	0	0	0	0	0	0	0	0	0	0	0	0	X	X	X	X	0	X	X	0
<i>Potamogeton richardsonii</i>	X	X	X	X	X	X	X	0	X	X	X	0	0	X	X	X	X	X	X	0
<i>Potamogeton robbinsii</i>	X	0	X	0	0	0	0	0	0	X	X	0	0	0	0	0	0	X	X	0
<i>Potamogeton zosteriformis</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	0	0	0	0	X	X	X
<i>Stuckenia pectinata</i>	X	X	X	0	X	X	X	0	X	X	X	0	X	X	X	0	X	0	X	X
<i>Utricularia vulgaris</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	0	X	X	X
<i>Vallisneria americana</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Zizania palustris</i>	0	0	0	0	X	0	X	0	0	X	X	0	X	X	X	0	X	X	X	X

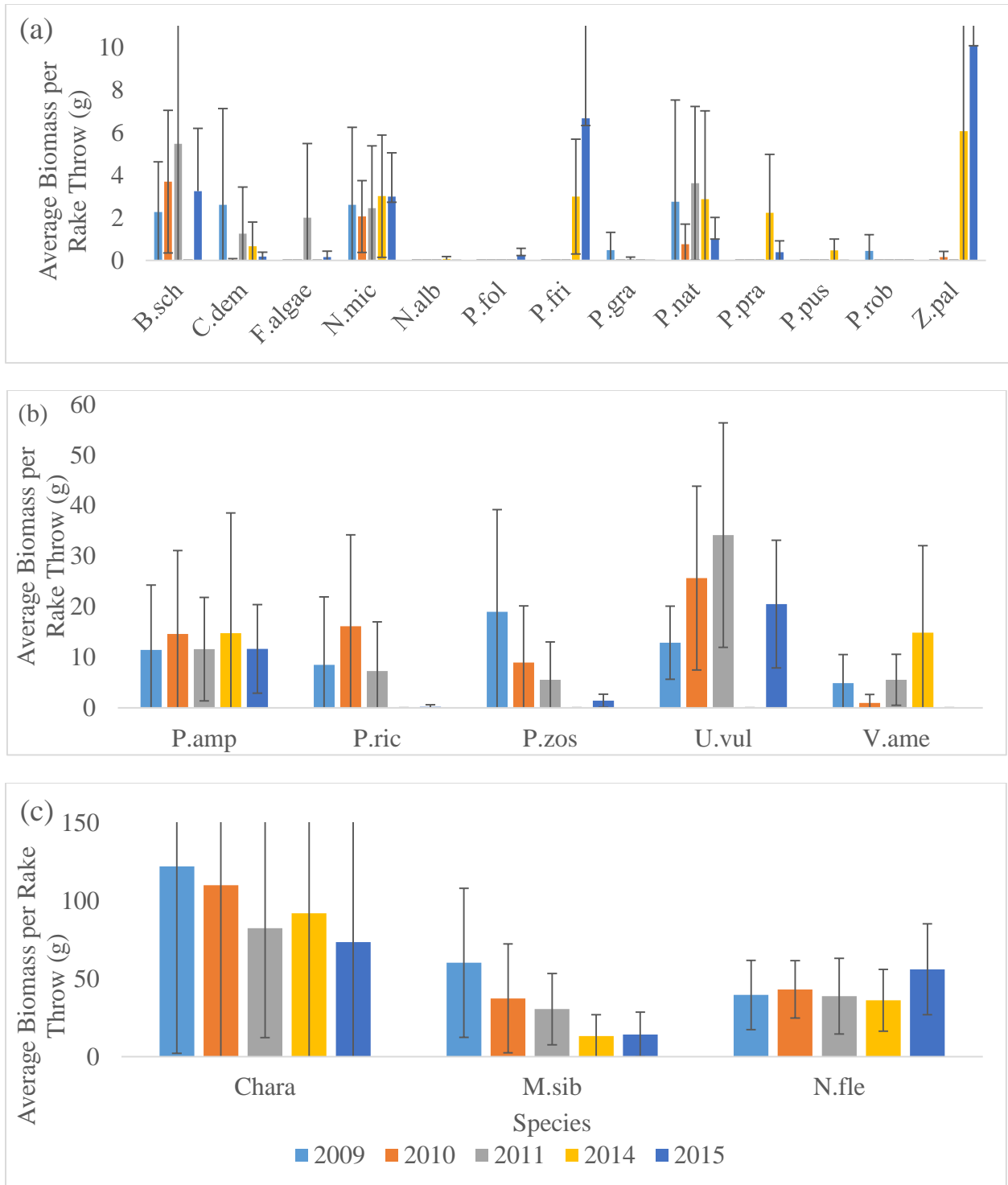


Figure 3.7: Average SAV species biomass (\pm SD) per year for bog sites, (a) y-axis ranges from 0-10 g, (b) y-axis ranges from 0-60 g, (c) y-axis ranges from 0-140 g. Species names are abbreviated with the first letter of the genus followed by the first three letters of the species names. Full genus and species names can be found in table 3.2.

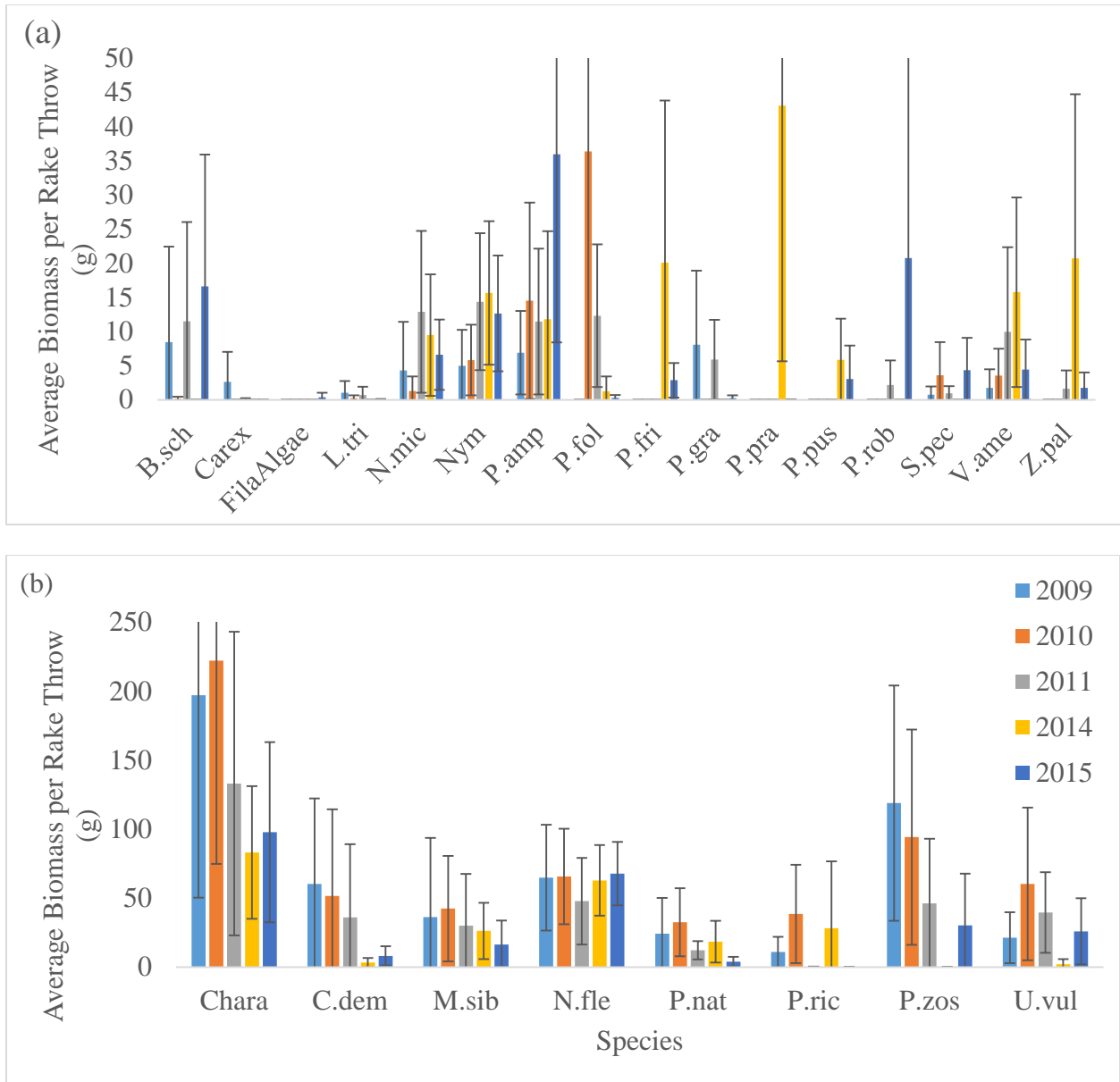


Figure 3.8: Average SAV biomass (\pm SD) for the isolated sites, (a) y-axis ranges from 0-50 g, (b) y-axis ranges from 0-250 g. Species names are abbreviated with the first letter of the genus followed by the first 3 letters of the species name. Full genus and species names can be found in table 3.2.

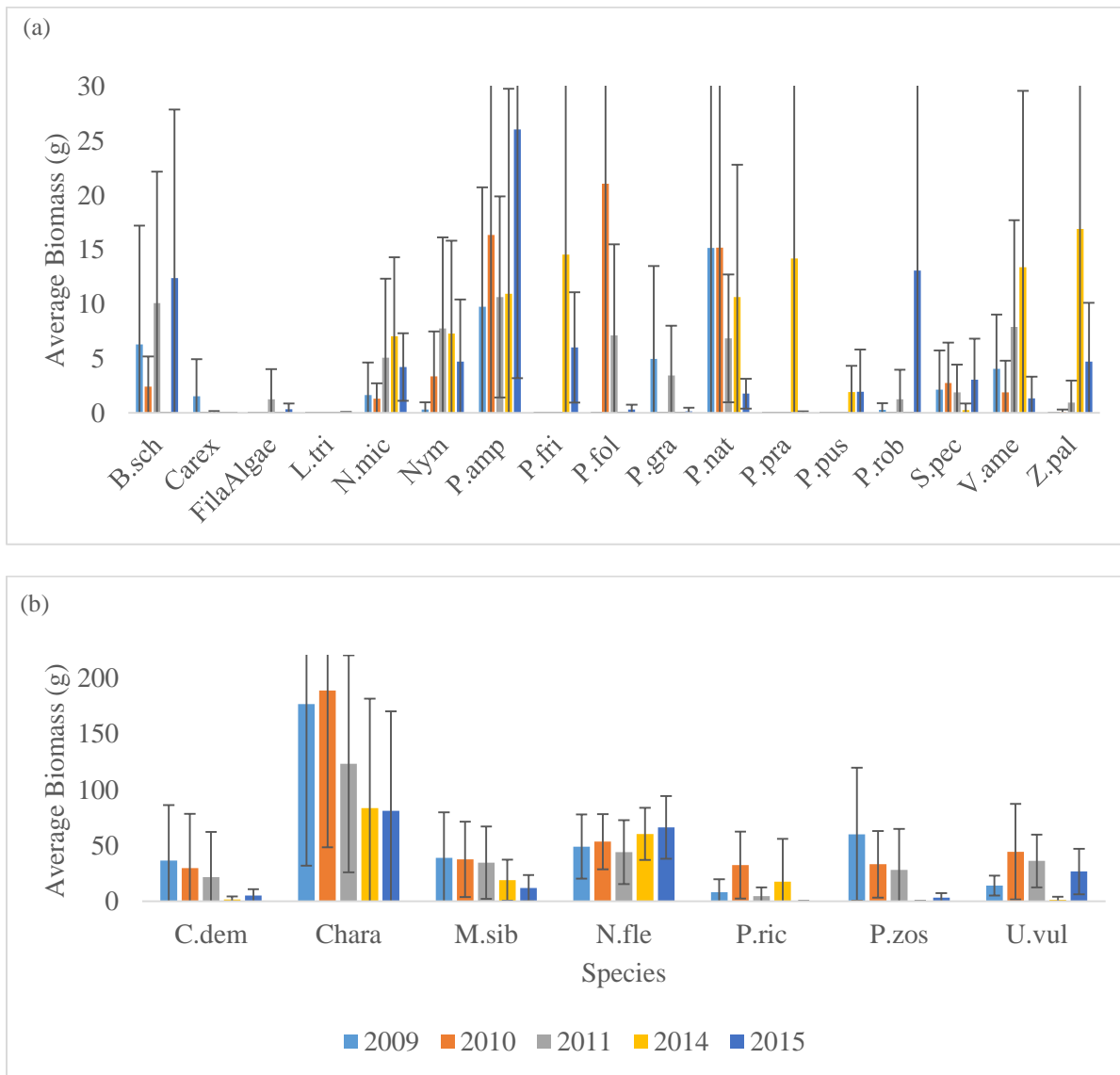


Figure 3.9: Average SAV biomass (\pm SD) for sites with fish, (a) y-axis ranges from 0-30, (b) y-axis ranges from 0-200. Species names are abbreviated with the first letter of the genus followed by the first 3 letters of the species name. Full genus and species names can be found in table 3.2.

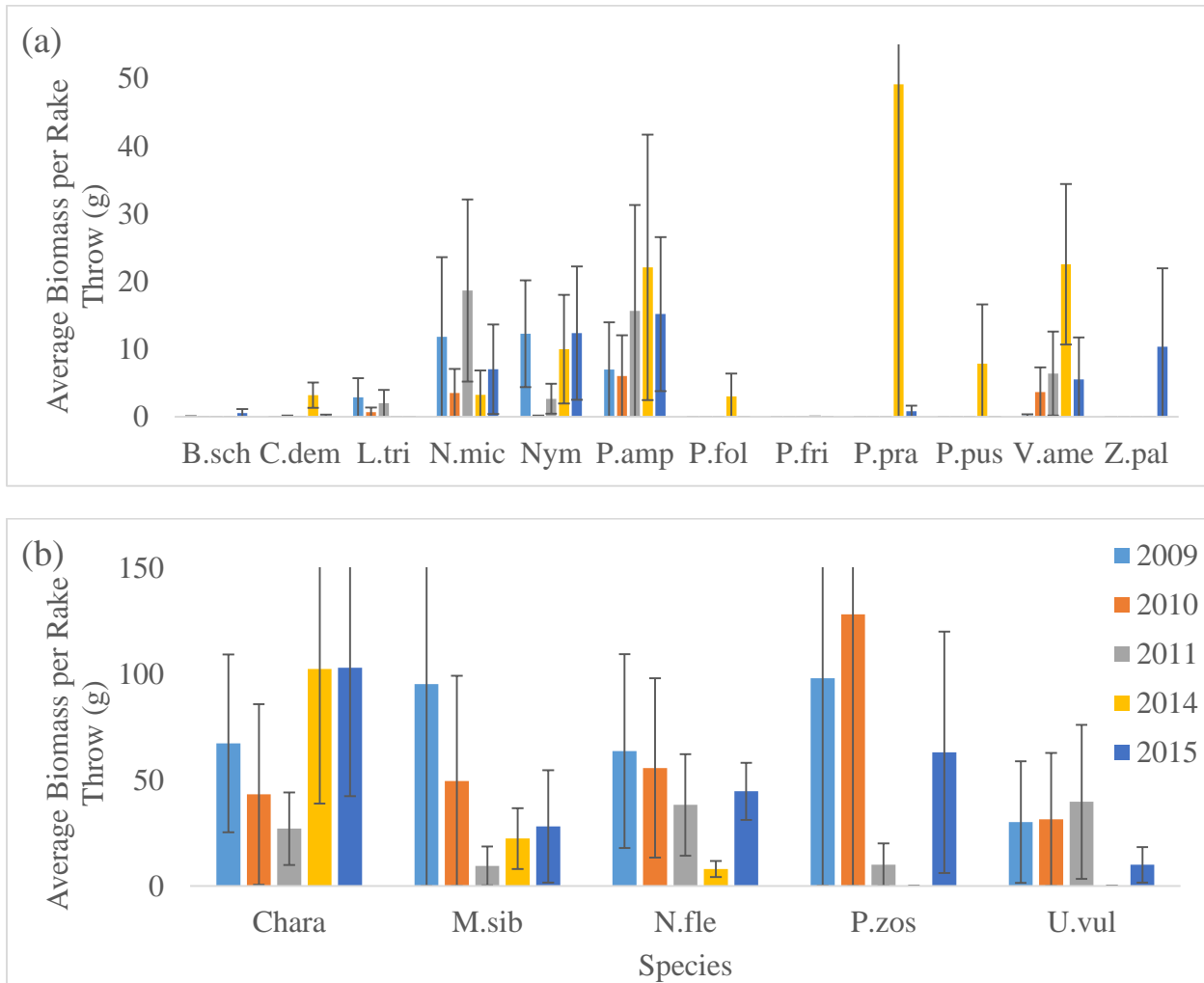


Figure 3.10: Average SAV biomass (\pm SD) for fishless sites, (a) y-axis ranges from 0-50, (b) y-axis ranges from 0-150. Species names are abbreviated with the first letter of the genus followed by the first 3 letters of the species name. Full genus and species names can be found in table 3.2.

Water quality parameters did not have a lot of variability with the highest variability in alkalinity and conductivity (table 3.3). Chl *a* concentrations were very low with highest average values in the bog sites of $5.10 \mu\text{g L}^{-1} \pm 5.4$. Phosphorus had the highest concentrations in the bog sites of $0.003 \text{ mg L}^{-1} \pm 0.002$. The pH of the sites averaged 7.7 ± 0.22 , (table 3.3). We found no

significant difference between bog sites and isolated sites for any of the water quality parameters measured ($p > 0.05$).

Table 3.3: Water quality parameters. Averages \pm standard deviation for each parameter measured. Units for each parameter are indicated in parenthesis next to the parameter variable.

Parameter	Averages \pm Standard Deviation					
	All Sites		Bog		Isolated	
Alkalinity (mg/l)	137.26 \pm	48.18	123.51 \pm	39.51	148.90 \pm	53.17
Ammonia-nitrogen (mg/l)	0.09 \pm	0.11	0.13 \pm	0.15	0.06 \pm	0.02
Chlorophyll a (ug/l)	5.10 \pm	5.38	6.53 \pm	7.54	3.90 \pm	2.16
Conductivity (uS/cm)	293.58 \pm	102.03	261.39 \pm	79.13	320.82 \pm	113.94
Dissolved Oxygen (mg/l)	8.25 \pm	1.73	9.36 \pm	1.23	7.31 \pm	1.54
Inorganic nitrogen (mg/l)	0.07 \pm	0.09	0.05 \pm	0.00	0.10 \pm	0.12
Nitrogen (mg/l)	1.09 \pm	0.41	1.29 \pm	0.49	0.91 \pm	0.22
Nitrate and Nitrite (mg/l)	1.04 \pm	0.31	1.21 \pm	0.34	0.89 \pm	0.21
Organic Carbon (mg/l)	17.10 \pm	8.05	22.79 \pm	7.51	12.28 \pm	4.71
Ortho Phosphate (mg/l)	0.00 \pm	0.00	0.01 \pm	0.00	0.00 \pm	0.00
pH	7.74 \pm	0.22	7.73 \pm	0.20	7.75 \pm	0.24
Phosphorus (mg/l)	0.02 \pm	0.01	0.03 \pm	0.02	0.02 \pm	0.01
Sulfate (mg/l)	0.32 \pm	0.25	0.23 \pm	0.21	0.37 \pm	0.26
Temperature ($^{\circ}$ C)	10.15 \pm	1.82	10.24	1.37	10.08 \pm	2.19
Turbidity (NTU)	4.09 \pm	5.19	6.15 \pm	7.23	2.36 \pm	1.02

NMS analysis recommended a final ordination with 3 axes, which yielded a stress value of 17.1, and a cumulative r^2 value of 0.773 representing 77.3% of the variation in the data set, with axis one representing 45.0% of the variation, axis two representing 15.5% and axis 3 representing 16.8% of the variation. Similar site score values will cluster together, values which are less similar will be further from one another. Upon examination of species correlations to the axis, we found no fish species correlated positively or negatively with any of the three axes in the NMS, and all r values for all species on all axis were between -0.500 and 0.500. We did find some SAV species to have r values above 0.500 and below -0.500 indicating strong relationships

with the axis. *Chara* spp. correlated negatively with the first axis (-0.770), as well as *Potamogeton zosteriformis* (-0.582) and total biomass (-0.837). *Myriophyllum sibiricum* correlated positively with the second axis (0.505), as well as *Utricularia vulgaris* (0.551). *Vallisneria americana* correlated negatively with the third axis with an r value of -0.583, and *Brasenia schreberi* correlated positively with the third axis with an r value of 0.557. This indicates that we do not see correlations between fish species and SAV species, but there are relationships between the SAV species. Those which correlated in the same direction on the same axis have a positive relationship while those which correlated in opposite directions on the same axis had a negative relationship. The MRPP revealed a significant difference between our sites with fish present and fishless sites ($A=0.010$, $p < 0.05$), a significant difference between our site locations, bog and isolated sites ($A=0.040$, $p < 0.05$), and a significant difference between years ($A=0.034$, $p < 0.05$). The convex hulls of the categorical variables drawn over the NMS ordination are displayed in figures 3.11-3.13. When we separated the bog and isolated sites data sets and looked for axis correlations within those data sets we did not find any significant correlations between SAV species and fish species, further indicating that there are no species specific relationships.

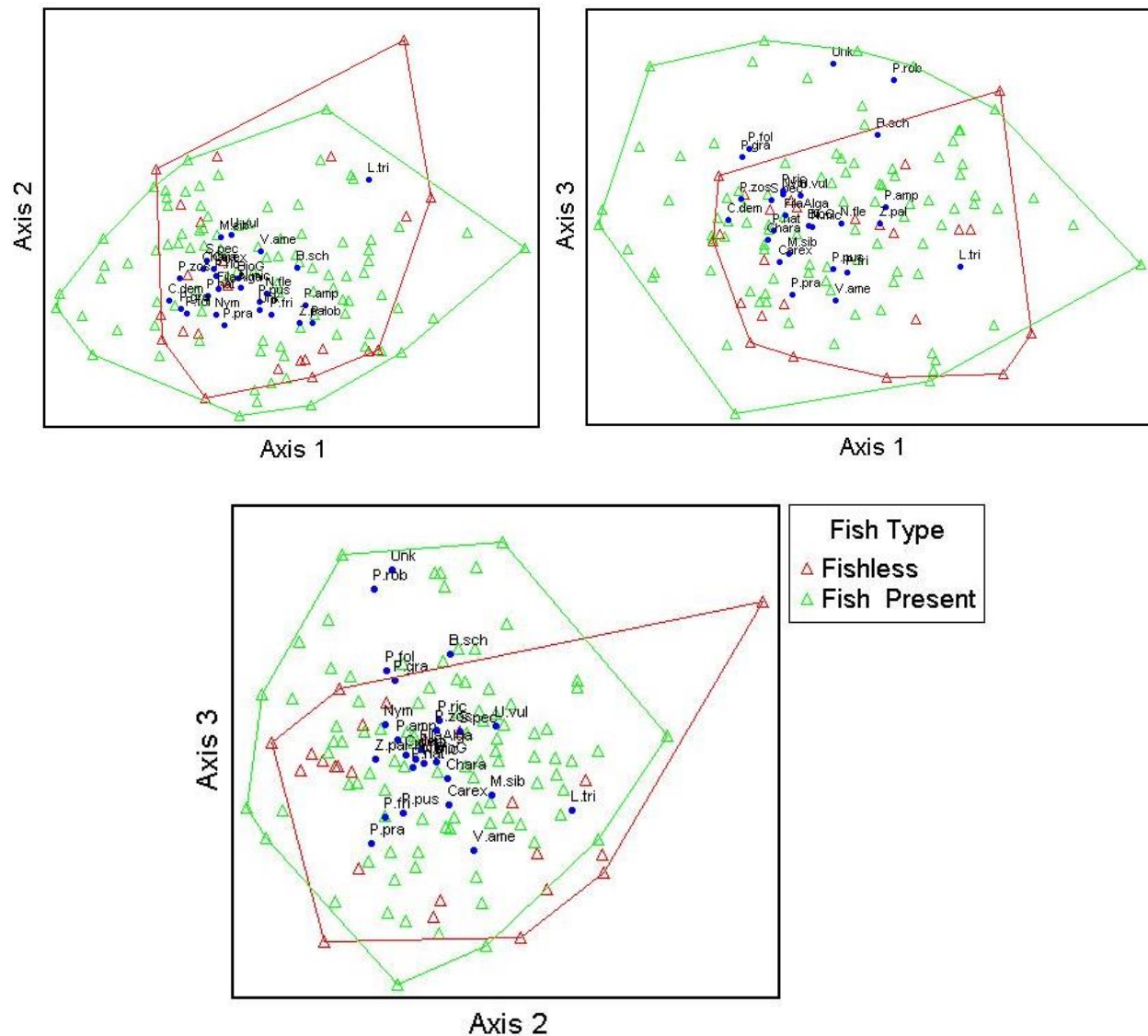


Figure 3.11: NMS ordination with all view of the 3 axes displayed, cumulative r^2 value of 0.773 representing 77.3% of the variation in the data set, with axis one representing 45.0% of the variation, axis two representing 15.5% and axis three representing 16.8% of the variation. Categorical variable convex hulls of fish type displayed. Site scores are represented with triangles, species scores of SAV species are represented with solid circles and labeled with abbreviations, full scientific names can be found in table 3.2. MRPP revealed fish presence or absence is significantly different ($A=0.010$, $p < 0.05$).

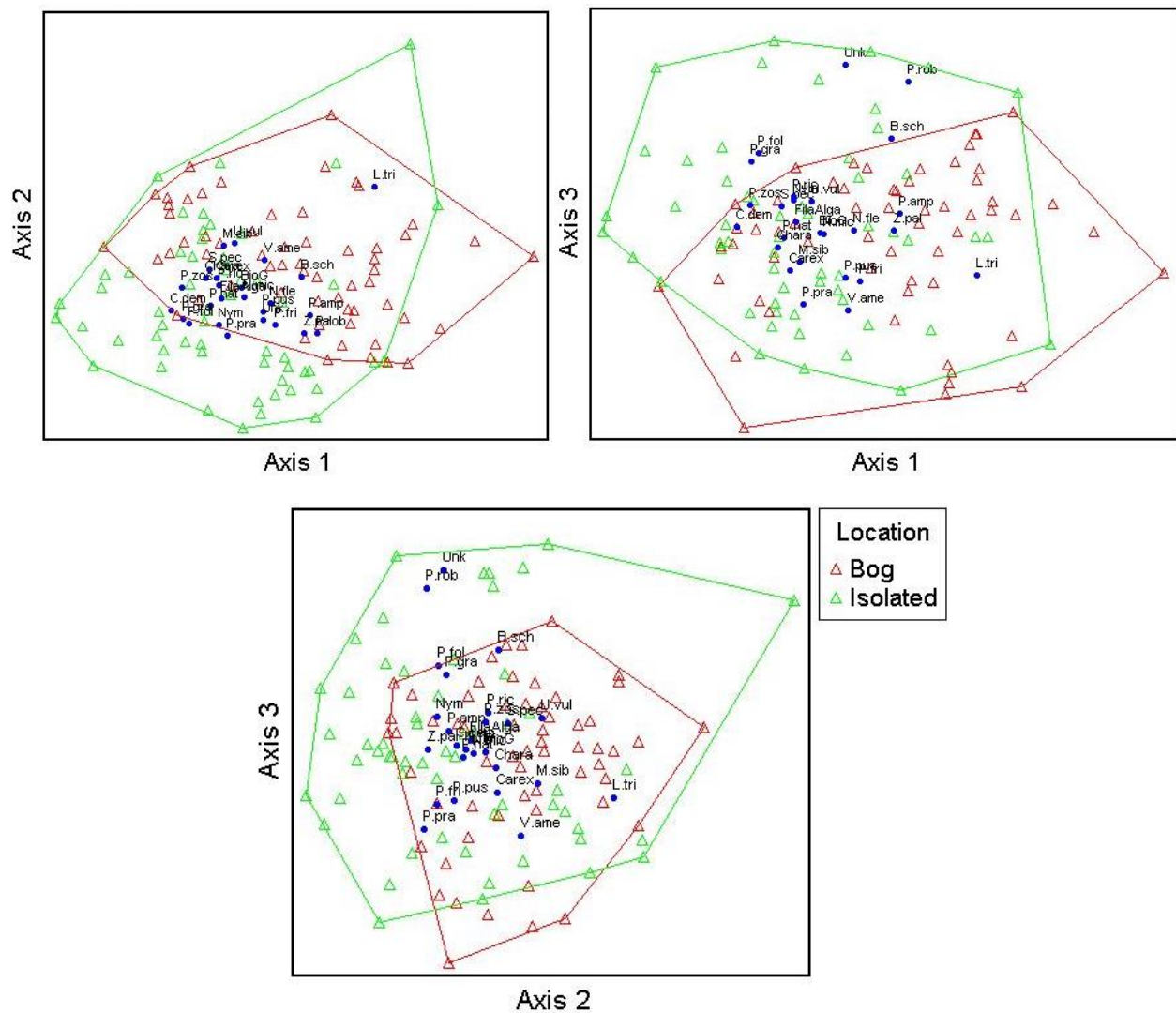


Figure 3.12: NMS ordination with all view of the 3 axis displayed, cumulative r^2 value of 0.773 representing 77.3% of the variation in the data set, with axis one representing 45.0% of the variation, axis two representing 15.5% and axis 3 representing 16.8% of the variation. Categorical variable convex hulls of location displayed. Site scores are represented with triangles, species scores of SAV species are represented with solid circles and labeled with abbreviations, full scientific names can be found in table 3.2. MRPP revealed location is significantly different ($A=0.040$, $p < 0.05$).

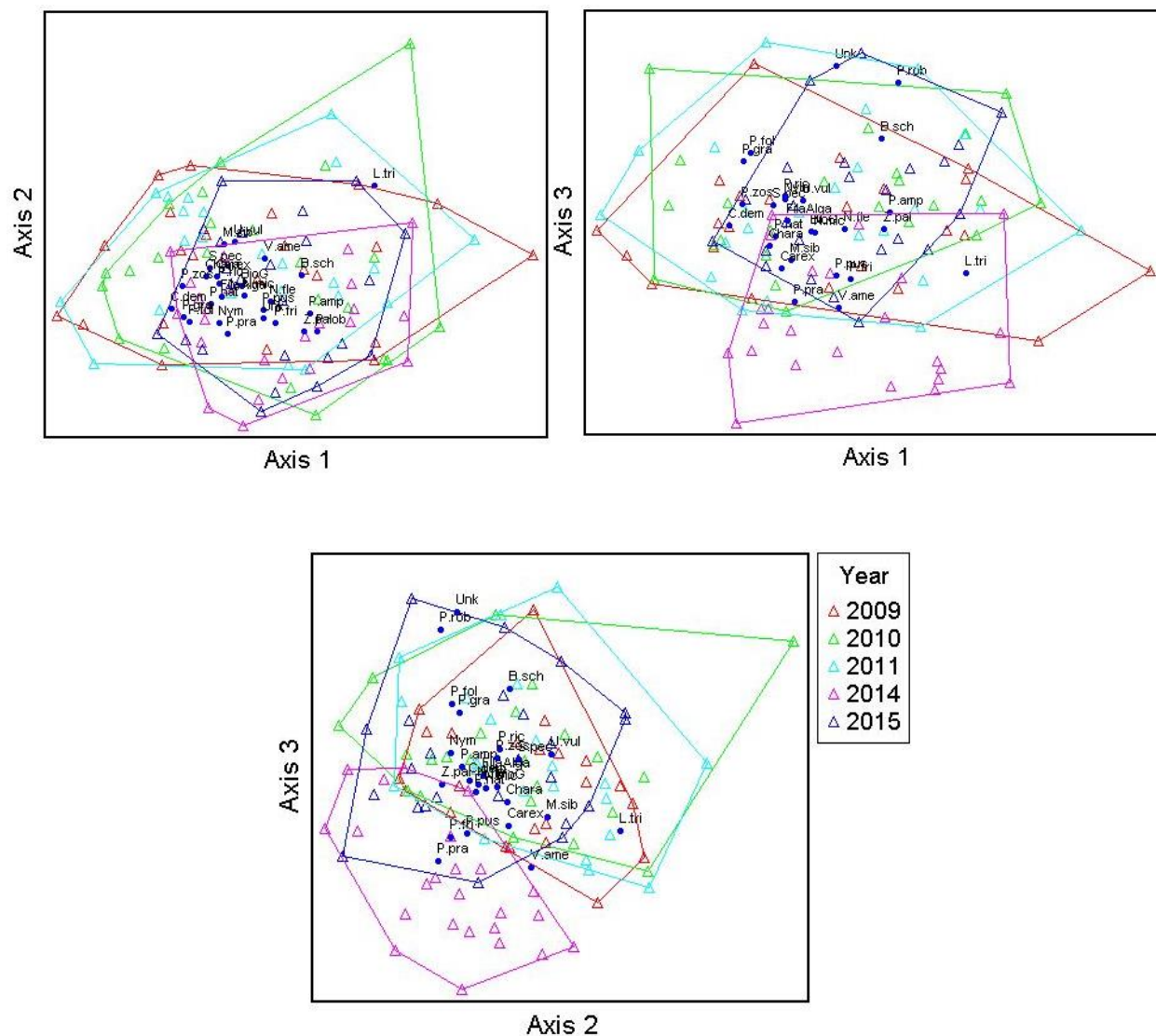


Figure 3.13: NMS ordination with all view of the 3 axis displayed, cumulative r^2 value of 0.773 representing 77.3% of the variation in the data set, with axis one representing 45.0% of the variation, axis two representing 15.5% and axis 3 representing 16.8% of the variation. Categorical variable convex hulls of year displayed. Site scores are represented with triangles, species scores of SAV species are represented with solid circles and labeled with abbreviations, full scientific names can be found in table 3.2. MRPP revealed year is significantly different ($A=0.034$, $p < 0.05$).

Discussion

We may have seen significant differences between our two site locations in fish biomass and species richness because our bog sites are located in closer proximity to the Red Lake River, and are more interconnected being surrounded by floating mat and quaking mats of vegetation. Sites which are interconnected and larger in size have a greater probability for fish or recolonization during overflow events (Herwig et al., 2010). It would take less intense rain events to create overland flow between these sites than our isolated sites because of their close proximity to one another and high water saturation of the surrounding landscape. At the furthest distance our bog sites were within 5.0 Km of one another while isolated sites at their furthest were within 25Km of one another. Shallow lake wetlands which have been impacted by development and farming in southern Minnesota, USA, have been observed to be colonized by fathead minnows (Zimmer et al., 2001; Zimmer et al., 2002). In addition, a study by Herwig et al. (2010), which encompassed regions of western and northern Minnesota, found a total 25 different species of fish inhabiting their study sites and average species richness of 5.6 in the northern prairie parkland region with all of the species found in our study also observed in their study. We found average species richness to range between 4-5 in our bog sites and from 2-4 in our isolated sites, indicating that in a pristine setting average species richness may be lower than in an impacted setting possibly because of limited human interactions and introductions of new species.

The SAV in our sites can also contribute to stability within the clear vegetated state. Macrophytes stabilize the clear state by providing hiding cover for zooplankton from fish predation (Zimmer et al., 2003; Scheffer, 2004). Perrow et al. (1999) found that in the presence of fish, the use of SAV significantly increased all species of *cladocerans*, indicating that

zooplankton do use SAV for hiding cover in the presence of fish. SAV biomass did not significantly change over the course of our study, nor did we see significant changes in species richness. SAV may be the main reason for why these sites have remained in the clear vegetated state, because SAV may have provided adequate hiding cover for zooplankton from fish predation, as well as stabilizing water clarity because of the continued phytoplankton grazing by zooplankton. Zimmer et al. (2003) found that the presence of fish decreased macrophyte abundance and species richness because it lead to increased turbidity when compared to sites without fish. We did not see an increase in SAV biomass when fish biomass decreased over the course of our study contrary to what Zimmer et al. (2003) found possibly because our sites did not have as much fish biomass. Zimmer et al. (2002) found an average of 50 kg per lake of fish biomass where in our study the highest fish biomass observed was 12.8 kg per lake. Species richness of SAV between our sites and Zimmer et al. (2003) were comparable (9), with similar species dominant such as *Chara* spp., *Ceretophyllum demersum*, *Myriophyllum sibiricum*, *Najas flexilis*, and *Potamogeton* spp. indicating that the composition of SAV species is similar in impacted and pristine sites in Minnesota. Clear lakes have more SAV biomass than turbid lakes because clear lakes have greater light penetration essential for plant growth (Jackson, 2003). Jackson (2003) found clear lakes to have an average of 4.14 kg m⁻² while turbid lakes had only 0.85 kg m⁻². This shows clear lakes harbor greater SAV biomass than turbid lakes.

This study did not find significant evidence of fish communities affecting SAV communities negatively or positively. We saw a decrease in fish biomass over time but did not see a similar decrease in SAV biomass or an increase in SAV biomass from 2009 to 2015, indicating that the presence or decrease of fish biomass does not induce changes in SAV biomass. The NMS analysis also revealed no significant correlations of SAV species with any of

the axes while some fish species correlated with the axes, indicating there are no species-specific relationships between the fish populations and SAV present in the lakes sampled in this study. Fish biomass and community composition are likely factors contributing to the stability of shallow lake state (Norlin et al., 2006; Roozen et al., 2007; Nolby et al., 2015). Sørensen et al. (2001) and Villena and Romo (2007) found a negative relationship between zooplankton and fish abundance, again indicating a direct relationship and food web interactions between fish and zooplankton, which then leads to cascading effects to phytoplankton, chlorophyll *a*, turbidity and SAV. The low fish biomass we found in our study seems to be contributing to stability in the clear vegetated state. Nolby et al. (2015) found that when fish biomass increased above 30 catch per unit effort (kg) (CPUE), the probability of a shallow lake to be in a clear water state greatly decreases. In 2009 we observed our highest fish biomass per lake (kg) of $7.7 \text{ kg} \pm 4.0$, which is well below the threshold for state change found by Nolby et al. (2015), and probably a contributing factor to why we see all of the lakes in our study in the clear state. We also found Chl *a* values for our sites averaging at $5.10 \text{ } \mu\text{gL}^{-1} \pm 5.3$ which is well below the threshold value of $60 \mu\text{gL}^{-1}$ where shallow lakes tend to switch from the clear vegetated state to the turbid un-vegetated state (Bayley et al., 2007). The low fish biomass found in our study sites contributes to these lakes staying in a clear vegetated state because high fish abundance leads to a decrease in zooplankton abundance and size which then leads to an increase in phytoplankton or Chl *a*, which increases the turbidity of the water column (O'Brien, 1979; Hanson and Butler, 1994a; Zimmer et al., 2001; Zimmer et al., 2003). Our low fish abundances probably indicate higher zooplankton abundances in our sites, and therefore a higher grazing pressure on phytoplankton, which results in the low Chl *a* measurement we saw in our sites. We also found only two benthivorous fish species in our study sites, *Ameiurus melas* (black bullhead) and *Ameiurus*

nebulosus (brown bullhead), which both occurred at < 0.5 kg per lake. Roosen et al. (2007) studied the effects of benthivorous carp (*Cyprinus carpio*) on lake turbidity in controlled enclosures (1.3 m X 1.05 m X 0.9 m). Each carp was approximately 0.225 kg, and was found to significantly increase suspended solids, phytoplankton, and turbidity of the water compared to enclosures without carp. The lakes in our study site were much larger than these enclosures, but only had about twice as much biomass of benthivorous fish present, indicating that the ratio of fish to lake area was probably too small to have a significant impact on lake turbidity.

Management Implications

Natural resource managers who wish to manage shallow lake wetlands for the clear vegetated alternative stable state may want to consider reducing fish biomass to levels not exceeding 12 kg per lake as seen in this study, as well as managing for SAV biomass ≥ 0.4 kg per rake throw. In our study sites, these levels of fish and SAV biomass and seemed to coincide with stability within the clear vegetated state from 2009-2015. Fish abundance increasing above 30 catch per unit effort (kg) can lead to a changes in state from clear to turbid, most likely due to predation on zooplankton, which leads to increases in phytoplankton populations, and more turbid lake water. SAV community composition and fish community composition in our pristine study sites are largely similar to impacted shallow lake wetland sites in Minnesota while the levels of abundance differed, with pristine sites having less fish biomass than impacted sites. Management therefore may need to be focused on managing the levels of abundance of SAV in shallow lake wetlands. Managing for greater SAV biomass and less fish biomass per lake may result in clearer lakes.

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CHAPTER 4: UNDERSTANDING THE RELATIONSHIPS BETWEEN SEDIMENT CHEMISTRY AND PLANT COMMUNITY COMPOSITION

Introduction

Shallow lake wetlands are extremely dynamic and complex systems, ranging from clear vegetated to turbid and un-vegetated states (Scheffer et al., 2001; Scheffer, 2004). The stability of these states depends upon many factors including fish communities and abundance (Jeppesen et al., 1999; Zimmer et al., 2001; Norlin et al., 2006; Roozen et al., 2007; Nolby et al., 2015), nitrogen and phosphorus concentrations and inputs (Cronk and Fennessy, 2001; Bayley et al., 2007, Alahuhta et al., 2012), and plant community composition (Carpenter and Lodge, 1986; Zimmer et al., 2001; Hanson et al., 2005). Understanding SAV community composition is important in the understanding of current state conditions, but many factors contribute to SAV community composition including current state stability, environmental factors (lake depth, precipitation, wave action, organic matter accumulation, land use) (Engel and Nicholas, 1994; Bayley et al., 2007; KISSOON et al., 2013), and abiotic factors (pH, alkalinity, salinity, turbidity, nutrients, etc.) (Stewart and Kantrud, 1972; Cowardin et al., 1979; Cronk and Fennessy, 2001; Reddy and DeLaune, 2008; KISSOON et al., 2015). The abiotic factors which make up sediment chemistry are very important in terms of contributing to plant community composition, and may largely be influenced by ground water and parent rock material (Moyle, 1945; Swartz and Gallup, 1978; Cowardin et al., 1979; Barko et al., 1991; Cronk and Fennessy, 2001; Loughheed et al., 2001; Hansel-Welch 2003; Capers et al., 2009; Alahuhta et al., 2012). Plants rooted in the sediment of shallow lakes obtain most of their nutrients from the sediment. Sediment characteristics and chemistry contribute largely to the abundance and biomass of supported vegetation and the species which can grow under the conditions exhibited by the sediment

(Barko et al., 1999; Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007). Plant communities can also change sediment characteristics by extracting nutrients during growth and reproduction, and returning nutrients to the sediment during senescence and decay (Carpenter and Lodge, 1986; Barko et al., 1991; Cronk and Fennessy, 2001).

Turbidity of the water column can be reduced or influenced by the presence of SAV, but turbidity can also influence the SAV species present (Engel and Nichols, 1994; Hansel-Welch et al., 2002; Scheffer, 2004). In Lake Christina (located in Minnesota, USA), the fish community was extirpated in an effort to increase water clarity, and return the lake to the clear-vegetated state from the turbid state. Only *Stuckenia pectinata* and *Najas* species were present in turbid conditions during pre-treatment observations, while *Najas* species, *Myriophyllum sibiricum* and *Rupia maritima* were found immediately following fish extirpation, indicating these species might be tolerant of slightly turbid conditions. *Chara vulgaris*, *Chara canescens*, *Potamogeton pectinatus* and *Potamogeton pussilus* were only found after the clear state became well established, indicating that these species are not tolerant of turbidity and probably need higher light penetration for survival (Hansel-Welch et al., 2003).

Many of the studies mentioned have only evaluated shallow lake plant communities in a landscape already altered by human activity. Understanding what conditions exist in a pristine setting is essential for successful restoration and conservation efforts, because studying already altered ecosystems does not provide adequate understanding of original conditions. Those studies which have assessed shallow lake ecology in an unaltered landscape have focused on phosphorus and nitrogen and water quality parameters but have yet to describe sediment chemistry and plant community species specific relationships (Bayley and Prather, 2003; Bayley et al., 2007). Understanding what elements and plant species are present in a pristine study site

will help guide baseline standards for wetland restoration projects, and will identify element concentrations which should be present in shallow lake wetlands and the plant communities associated with them. The objectives of this study were to 1) Refine baseline chemical, nutrient, and biological data for shallow lakes on the Red Lake Reservation and 2) Evaluate how sediment chemistry impacts submerged aquatic vegetation and emergent vegetation community composition in pristine shallow lakes.

Methods

Study Area

Our study site was located in the northwest region of Minnesota, U.S.A, on Red Lake Nation Indian Reservation land (47.8764° N, 95.0169° W). Average low temperatures of -15.4 °C occur during January, while average high temperatures of 26.8 °C occur during July annually. Highest average annual rainfall occurs during the month of June with about 11.0 cm of precipitation, while February is the driest month with only 2.0 cm of precipitation (NOAA, 2013). Our study site, Red Lake Nation Indian Reservation, is unique because the land has been under Native American jurisdiction. It is one of the few closed reservations within the United States, meaning access by non-tribal members is restricted. As a result, the land under the jurisdiction of Red Lake Indian Reservation has experienced limited human development, with no industry or agricultural practices within its boundary (Meyer, 1992; Treuer, 2010), making it as pristine a study area as possible in this region of the United States and an ideal area to collect baseline data on conditions of shallow lake wetlands in an un-impacted landscape. The wetlands chosen for this study are in the clear vegetated state and have never undergone state change during the monitoring period by Red Lake Department of Natural Resources from 2009-2015. These data will help managers to complete more successful restoration and mitigation projects,

to better represent naturally occurring wetlands, and understand impacts from climate change, because the wetlands provide an example of shallow lake ecology with very low human influences.

Sampling Strategy

SAV was collected using a rake throw technique. A common garden rake with a dual sided head was thrown out of both sides of the canoe, dredged along the bottom, and pulled up. The plant matter collected on the rake head was weighed and the percentage of each species of plant was estimated. Sampling took place along 4 transects with 5 sampling points spaced equidistant along each transect for a total of 20 sampling points, and 40 rake throws per lake. Emergent vegetation was sampled twice at the starting and ending points of each SAV transect. A 0.5 m X 0.5 m Daubenmire frame was used to sample the percent composition of emergent vegetation, with 1 m of the edge of the open water of each shallow lake, for a total of 16 random emergent vegetation sampling locations. Sampling of both SAV and emergent vegetation occurred at the same time, in the same 24 shallow lake wetland sites, from late June to early August in 2014 and 2015.

Sediment samples were collected by taking a grab sample of sediment from the top 0.5m of sediment at 8 random locations throughout each lake, 4 where SAV was also taken and 4 where emergent vegetation was taken. Samples were placed in airtight plastic bags and transported at 0-4 °C to the laboratory, where they were then transferred to paper bags, and dried to a constant dry weight at 60 °C, crushed with a porcelain mortar and pestle to homogenize the sample. Sediment samples were then analyzed for 61 elements with an Inductively Coupled Plasma Mass Spectrometry after they had been liquefied with Aqua Regia Digestion by Bureau Veritas Commodities Canada, Accredited Laboratory (<http://acmelab.com>). Detection limits for

elements are listed in table 4.1. Recovery rates were determined using the expected and measured values of a soil reference standard (OREAS45EA). We measured organic matter (OM) content of the sediment using loss on ignition techniques. Sediment was ground to homogenize the sample, a subsample taken with a NCR-13 5 gram scoop, and then was dried in a muffle furnace for 2 hours at 105°C to remove any moisture left within the sample. The sample was then placed in the muffle furnace for an additional 2 hours at 360°C to burn off organic matter. Samples were weighed and percent organic matter of the sample was calculated based on initial and final weights (USDA, 2011).

Water quality parameters were measured from a single dip sample in all 24 shallow lake sites, in the center of each lake. We used multi-probe models YSI 6920 V2-2 and YSI 6920 V2-2 to measure water temperature, conductivity, pH, and dissolved oxygen. We also measured turbidity in-house using USEPA method 180.1 with a HACH 2100Q turbidity meter. Alkalinity was measured in-house using HACH method 8221. The following parameters were analyzed by RMB Environmental Laboratories in Detroit Lakes, MN followed by their EPA method number; Total phosphorus (TP): EPA 365.3, Orthophosphate (OP): EPA 365.3, Total Dissolved Phosphorus (TDP): EPA 365.3, Inorganic nitrogen (nitrate & nitrite nitrogen) (listed as N+N or N2N3): EPA353.2 Rev 2.0, Total Kjeldahl Nitrogen (TKN): EPA 351.2 Rev 2.0, Ammonia-nitrogen (NH3): EPA 350.1 Rev 2.0, Sulfate (SO4): ASTM D516-07, Dissolved Organic Carbon (DOC): SM5310 C-96.

Table 4.1: Detection limits for elements from ICP-MS analysis

Element	$\mu\text{mole/g}$	Element	$\mu\text{mole/g}$
Ag	1.85E-05	Na	0.043499
Al	0.370631	Nb	0.000215
As	0.001335	Ni	0.001704
Au	1.02E-06	P	0.032286
B	1.850139	Pb	4.83E-05
Ba	0.003641	Pd	9.4E-05
Be	0.011096	Pt	1.03E-05
Bi	9.57E-05	Rb	0.00117
Ca	0.249513	Re	5.37E-06
Cd	8.9E-05	S	0.62383
Ce	0.000714	Sb	0.000164
Co	0.001697	Sc	0.002224
Cr	0.009616	Se	0.001266
Cs	0.00015	Sn	0.000842
Cu	0.000157	Sr	0.005706
Fe	0.179067	Ta	0.000276
Ga	0.001434	Te	0.000157
Ge	0.001377	Th	0.000431
Hf	0.000112	Ti	0.020891
Hg	2.49E-05	Tl	9.79E-05
In	0.000174	U	0.00042
K	0.255766	V	0.039261
La	0.003599	W	0.000544
Li	0.014409	Y	0.000112
Mg	0.411438	Zn	0.00153
Mn	0.018202	Zr	0.001096
Mo	0.000104		

Statistical Methods

All data values were averaged by lake prior to the start of any statistical analysis. We used multivariate statistical techniques to assess further if there were relationships between plant communities and sediment chemistry using non-metric multidimensional scaling (NMS) in PC-

ORD 6 (McCune and Mefford, 2001) as described by Kruskal (1964) and Mather (1976), using the Sørensen distance measure, with the “slow and thorough” procedure, and using random starting position and 250 runs with real and randomized data. NMS was chosen as the statistical technique because it is useful for finding relationships in multivariate ecological data and able to assess non-linear data sets with different scales of measurement (McCune and Grace, 2002). Prior to NMS, SAV and emergent plant species percent composition data were 0.25 power transformed to relativize the data in order to account for differences in magnitude of species sample masses (McCune and Grace, 2002). Emergent and open water sediment element concentrations were log transformed to relativize the data set and account for differences in range and magnitudes of scale within the data set. We assessed species specific relationships using Pearson and Kendall correlations with Ordination axes. We then used Multi-response permutation procedures (MRPP), using the Sørensen distance measure, to test for significance of our categorical variables; year and location (Mielke and Berry, 2001). We chose not to use a RDA analysis because we were interested in exploring the data set, and discovering which elements correlated with which plant species. Using an RDA analysis would have limited the environmental data matrix to 4-5 elements. We were uncomfortable limiting the element matrix to that extent because we are unsure which elements in our sediment data are most important for plant community composition. Using an NMS ordination and evaluating the Pearson and Kendall correlations with ordination axes enabled us to explore which elements are most important for which plant species. We compared those elements and species which showed significant correlations with the axes to Kisson et al.’s (2015) to see if trends existed among a wider range of element concentrations using Pearson’s correlation coefficients.

Results

The SAV consisted of *Ceratophyllum demersum*, *Chara* spp., *Myriophyllum sibiricum*, *Najas flexilis*, *Nuphar microphylla*, *Nymphaea alba*, *Potamogeton amplifolius*, *Potamogeton friesii*, *Potamogeton natans*, *Potamogeton praelongus*, *Vallisneria americana*, *Zizania palustris* in both sampling locations (bog and isolated) in all years of the study (table 4.2). Bog sites were characterized by dominant species of *Chara* spp. (average of 18% of composition), *Myriophyllum sibiricum* (5%), and *Najas flexilis* (26%) (fig. 4.1). Isolated sites were characterized with dominant species of *Chara* spp. (average of 15% of composition), *Myriophyllum sibiricum* (30%), *Najas flexilis* (3%), and *Potamogeton natans* (3%) with averages (fig. 4.2). In the emergent vegetation *Alnus* spp., *Asclepias incarnatae*, *Aster* spp., *Athyrium filix-femina*, *Betula pumila*, *Calla palustris*, *Carex laeviconica*, *Carex retrorsa*, *Carex* spp., *Dulichium arundinaceum*, *Eleocharis* spp., *Eriophorum* spp., *Eupatorium maculatum*, *Galium* spp., *Impatiens capensis*, and *Iris versicolor* occurred in both bog and isolated sites in all years of the study (table 4.3). *Chamaedaphne calyculata* was found exclusively in the isolated sites in 2014, while *Equisetum arvense* and *Glyceria* spp. were found exclusively in the isolated sites in 2015, and *Eupatorium perfoliatum* was found exclusively in the isolated sites both years of the study. *Larix laricina* was the only species found exclusively in the bog sites. The emergent vegetation in the bog sites was characterized with dominant species of *Typha* spp. (average of 21% composition), *Athyrium filix-femina* (15%), and *Carex laeviconica* (10%) (fig. 4.3). The isolated sites were characterized by dominant species including *Athyrium filix-femina* (averages of 10% composition), *Carex laeviconica* (11%), *Carex* spp. (16%), *Eleocharis* spp. (13%), and *Zizania palustris* (7%) (fig. 4.4).

Table 4.2: Presence absence of SAV species in bog and isolated sites in 2014 and 2015

Scientific Name	2014		2015	
	Bog	Isolated	Bog	Isolated
<i>Brasenia schreberi</i>	0	0	X	X
<i>Carex</i> spp.	0	0	0	0
<i>Ceratophyllum demersum</i>	X	X	X	X
<i>Chara</i> spp.	X	X	X	X
<i>Filamentous algae</i>	0	0	X	X
<i>Lemna trisulca</i>	0	0	0	X
<i>Myriophyllum sibiricum</i>	X	X	X	X
<i>Najas flexilis</i>	X	X	X	X
<i>Nuphar microphylla</i>	X	X	X	X
<i>Nymphaea alba</i>	X	X	X	X
<i>Potamogeton amplifolius</i>	X	X	X	X
<i>Potamogeton crispus</i>	X	0	0	0
<i>Potamogeton friesii</i>	X	X	X	X
<i>Potamogeton foliosus</i>	X	0	X	X
<i>Potamogeton gramineus</i>	0	0	0	X
<i>Potamogeton natans</i>	X	X	X	X
<i>Potamogeton praelongus</i>	X	X	X	X
<i>Potamogeton pusillus</i>	X	X	0	X
<i>Potamogeton richardsonii</i>	X	X	0	X
<i>Potamogeton robbinsii</i>	0	0	0	X
<i>Potamogeton zosteriformis</i>	0	0	X	X
<i>Stuckenia pectinata</i>	0	X	X	X
<i>Utricularia vulgaris</i>	0	X	X	X
Unknown	0	0	0	X
<i>Vallisneria americana</i>	X	X	X	X
<i>Zizania palustris</i>	X	X	X	X

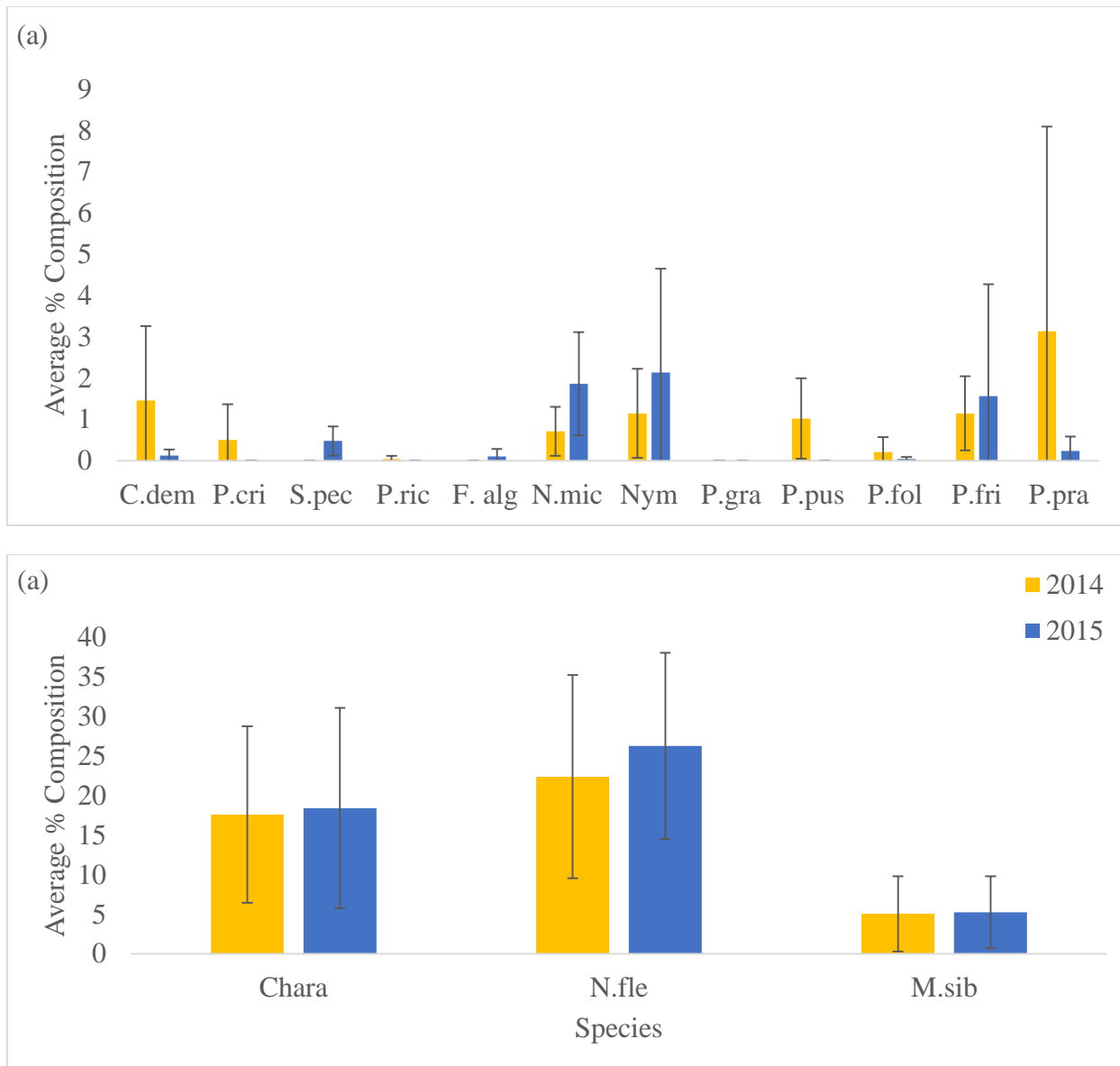


Figure 4.1: Average percent composition (\pm SD) of SAV species in 12 bog site locations per year, (a) y-axis ranges from 0-9 % composition, (b) y-axis ranges from 0-40 % percent composition. Species names are abbreviated with the first letter of the genus followed by the first 3 letters of the species name. Full scientific names can be found in table 4.1.

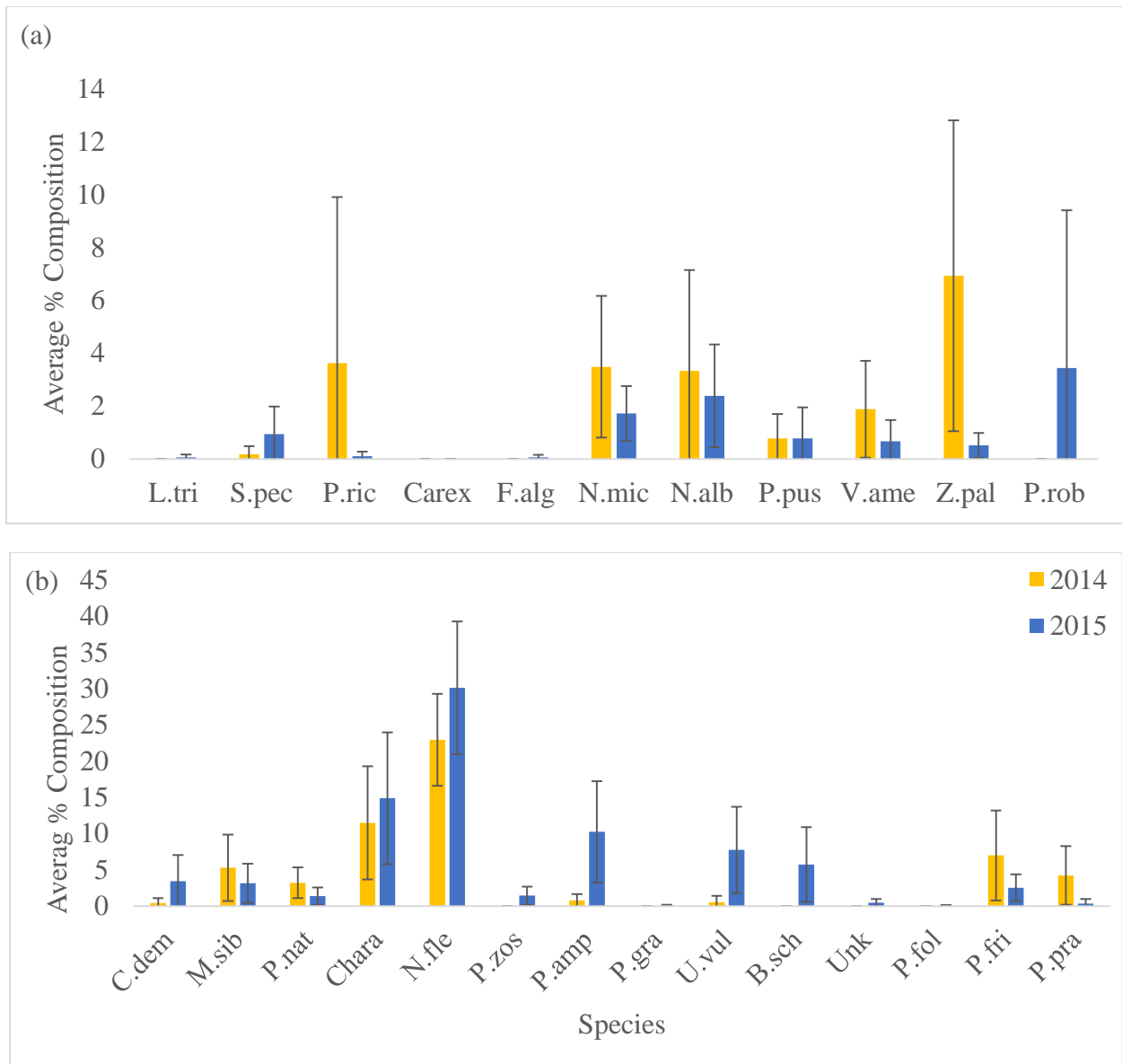


Figure 4.2: Average percent composition (\pm SD) of SAV species in 12 isolated site locations per year, (a) y-axis ranges from 0-14 % composition, (b) y-axis ranges from 0-45 % composition. Species names are abbreviated with the first letter of the genus followed by the first 3 letters of the species name. Full scientific names can be found in table 4.1.

Table 4.3: Presence absence of emergent vegetation in bog and isolated sites per year

Scientific Name	2014		2015	
	Bog	Isolated	Bog	Isolated
<i>Alnus</i> spp.	X	X	X	X
<i>Asclepias incarnata</i>	X	X	X	X
<i>Aster</i> spp.	X	X	X	X
<i>Athyrium filix-femina</i>	X	X	X	X
<i>Betula pumila</i>	X	X	X	X
<i>Calla palustris</i>	X	X	X	X
<i>Carex laeviconica</i>	X	X	X	X
<i>Carex retrorsa</i>	X	X	X	X
<i>Carex</i> spp.	X	X	X	X
<i>Chamaedaphne calyculata</i>	0	X	0	0
<i>Cicuta bulbifera</i>	0	X	X	X
<i>Cirsium muticum</i>	0	X	0	0
<i>Cornus sericea</i>	0	X	0	0
<i>Dulichium arundinaceum</i>	X	X	X	X
<i>Eleocharis</i> spp.	X	X	X	X
<i>Equisetum arvense</i>	0	0	0	X
<i>Eriophorum</i> spp.	X	X	X	X
<i>Eupatorium maculatum</i>	X	X	X	X
<i>Eupatorium perfoliatum</i>	0	X	0	X
<i>Galium</i> spp.	X	X	X	X
<i>Glyceria</i> spp.	0	0	0	X
<i>Impatiens capensis</i>	X	X	X	X
<i>Iris versicolor</i>	X	X	X	X
<i>Larix laricina</i>	X	0	0	0
<i>Leersia oryzoides</i>	0	0	X	X
<i>Lycopus</i> spp.	0	0	X	X
<i>Lysimachia</i> spp.	0	0	X	X
<i>Menyanthes trifoliata</i>	0	0	0	X
<i>Nuphar microphylla</i>	X	X	0	0
<i>Nymphaea odorata</i>	0	X	0	X
<i>Phalaris arundinacea</i>	0	X	X	X
<i>Phragmites</i> spp.	X	X	X	X
<i>Potamogeton natans</i>	X	X	0	0
<i>Potentilla palustris</i>	X	X	X	X

Table 4.3: Presence absence of emergent vegetation in bog and isolated sites per year (continued)

Scientific Name	2014		2015	
	Bog	Isolated	Bog	Isolated
<i>Sagittaria cuneata</i>	X	X	X	X
<i>Salix exigua</i>	0	X	X	0
<i>Salix pedicellaris</i>	0	0	X	X
<i>Salix</i> spp.	X	X	X	X
<i>Sarracenia purpurea</i>	0	0	0	X
<i>Scirpus acutus</i>	X	X	X	X
<i>Scirpus validus</i>	X	X	0	X
<i>Scutellaria lateriflora</i>	X	X	X	X
<i>Sparganium</i> spp.	0	0	0	X
<i>Triadenum fraseri</i>	X	X	X	X
<i>Typha angustifolia</i>	X	X	X	0
<i>Typha latifolia</i>	X	X	X	X
<i>Typha</i> spp.	X	X	X	X
<i>Verbena hastata</i>	0	X	0	0
<i>Zizania palustris</i>	0	X	X	X

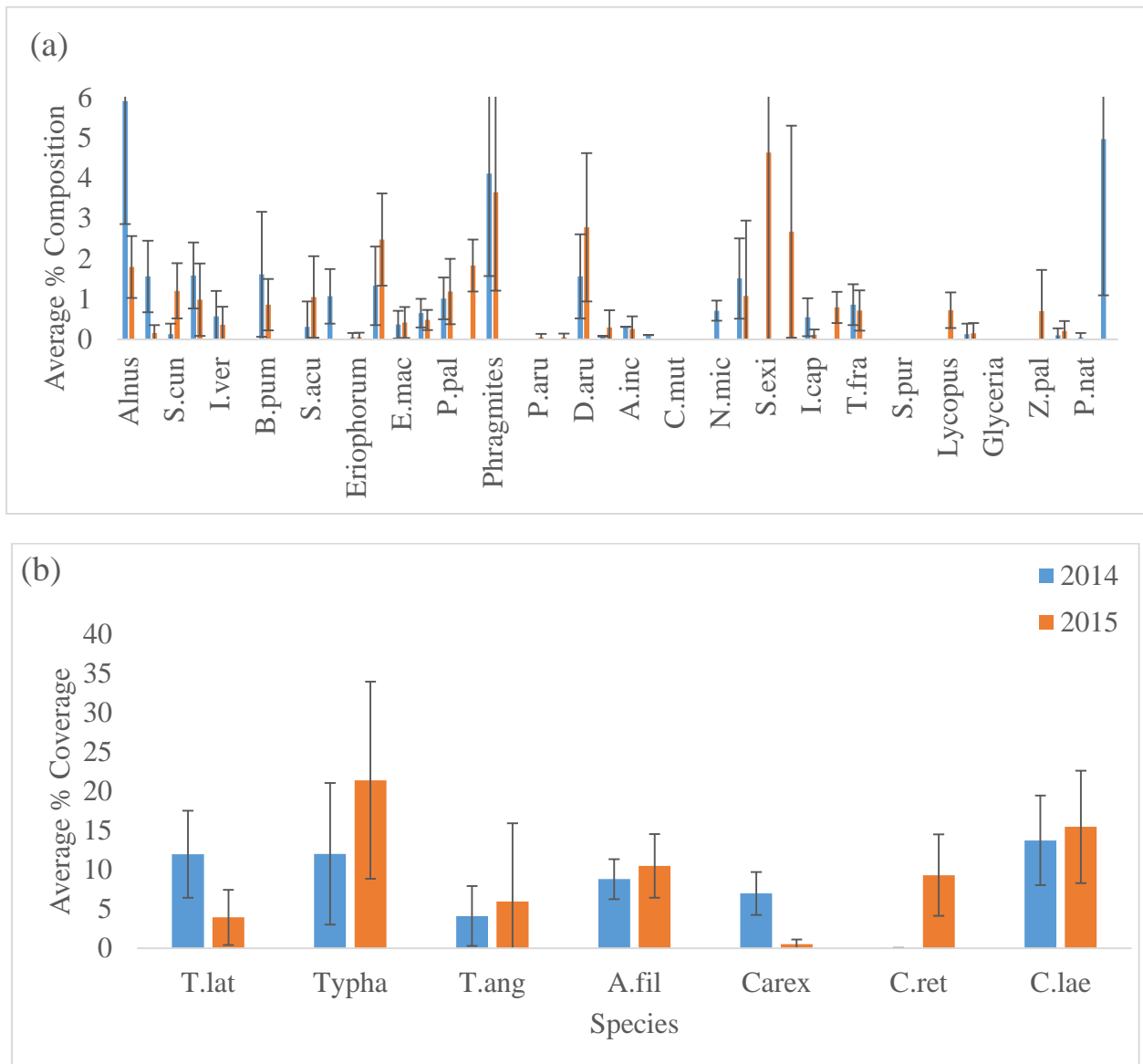


Figure 4.3: Average percent composition (\pm SD) of emergent species in 12 bog site locations per year, (a) y-axis ranges from 0-6% composition, (b) y-axis ranges from 0-40% composition. Species names are abbreviated with the first letter of the genus followed by the first 3 letters of the species name. Full scientific names can be found in table 4.3.

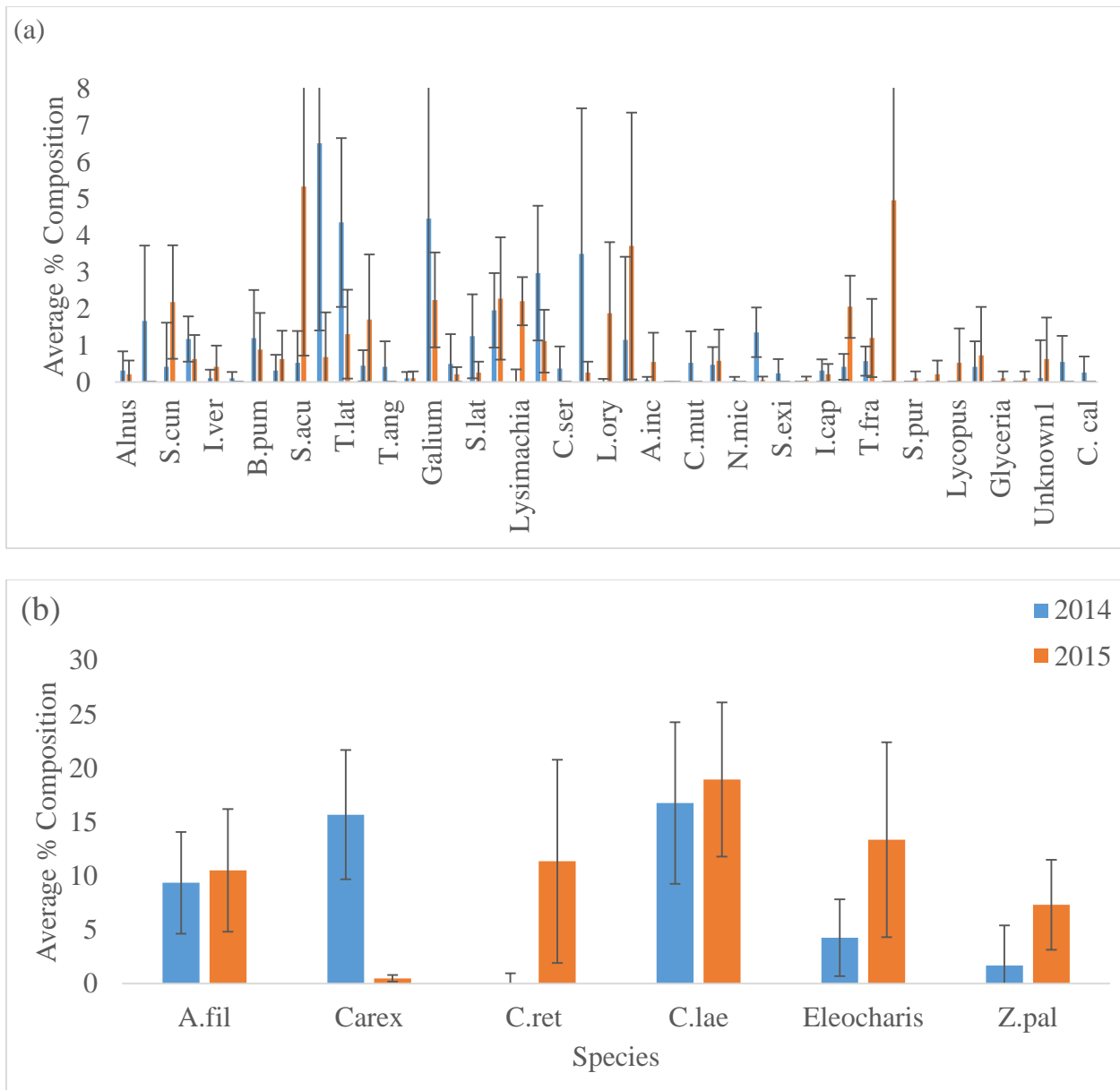


Figure 4.4: Average percent composition (\pm SD) of emergent species in 12 isolated site locations per year, (a) y-axis ranges from 0-8% composition, (b) y-axis ranges from 0-30% composition. Species names are abbreviated with the first letter of the genus followed by the first 3 letters of the species name. Full scientific names can be found in table 4.3.

For our sediment analysis, we removed elements which had concentrations at or below the detections limits from the analysis. For the sediment taken beneath the open water zone of the lakes in conjunction with SAV sampling we used data on Ag, Al, As, Ba, Bi, Ca, Cd, Ce, Co,

Cr, Cs, Cu, Fe, Ga, Hg, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Rb, S, Sb, Sc, Se, Sn, Sr, Th, Ti, Tl, U, V, Y, Zn, and Zr. The elements with the highest concentrations were Ca, Mg, Mn, and S, while the lowest concentrations were observed for Ag, As, Bi, Cd, Cs, Hg, Mo, Nb, Sb, Sn, Th, Tl, and U (table 4.4). MRPP showed no significant differences between years ($A = -0.009$, $p = 0.93$) but a significant difference between site locations ($A = 0.0$, $p < 0.01$) in the open water sediment data. For the sediment sampled within the emergent vegetation we used Ag, Al, As, Ba, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Hg, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Rb, S, Sb, Sc, Se, Sr, Tl, U, V, Y, Zn, and Zr. Here, elements with the highest sediment concentrations Ca, Mg, Mn, and S, while elements with the lowest concentrations were Ag, Bi, Cd, Cs, Ga, Hg, Mo, Nb, Sb, Sc, Se, Tl, U, and Zr (table 4.5).

Table 4.4: Average element concentration \pm standard deviations for sediment element concentrations of the open water zone of the shallow lake wetlands. Concentrations are presented in $\mu\text{mol g}^{-1}$ unless indicated by † for nmol g^{-1}

Element	Average \pm Standard Deviation							
	Bog				Isolated			
	2014		2015		2014		2015	
Ag†	0.23 \pm	0.07	0.24 \pm	0.05	0.30 \pm	0.09	0.29 \pm	0.10
Al	9.43 \pm	2.41	9.58 \pm	2.22	10.93 \pm	3.29	11.10 \pm	4.39
As†	42.44 \pm	13.33	43.49 \pm	12.32	36.90 \pm	11.38	37.68 \pm	14.27
Ba	0.81 \pm	0.32	0.90 \pm	0.35	0.99 \pm	0.32	1.01 \pm	0.28
Bi†	0.64 \pm	0.17	0.60 \pm	0.13	0.55 \pm	0.12	0.62 \pm	0.29
Ca	69.33 \pm	49.28	65.23 \pm	34.44	119.29 \pm	98.21	130.35 \pm	122.54
Cd†	4.25 \pm	1.29	4.11 \pm	0.80	3.59 \pm	1.02	3.89 \pm	1.75
Ce	0.05 \pm	0.01	0.05 \pm	0.01	0.08 \pm	0.03	0.08 \pm	0.03
Co	0.04 \pm	0.01	0.04 \pm	0.01	0.04 \pm	0.01	0.04 \pm	0.02
Cr	0.10 \pm	0.05	0.09 \pm	0.02	0.12 \pm	0.04	0.12 \pm	0.04
Cs†	2.85 \pm	0.78	2.61 \pm	0.54	2.63 \pm	0.84	2.72 \pm	1.06
Cu	0.09 \pm	0.02	0.09 \pm	0.01	0.13 \pm	0.04	0.12 \pm	0.05
Fe	9.62 \pm	3.40	10.85 \pm	4.22	10.05 \pm	3.07	10.20 \pm	4.09
Ga	0.01 \pm	0.00	0.01 \pm	0.00	0.01 \pm	0.00	0.01 \pm	0.01
Hg†	0.39 \pm	0.08	0.44 \pm	0.08	0.34 \pm	0.07	0.33 \pm	0.10
K	2.66 \pm	1.41	3.92 \pm	2.49	4.53 \pm	2.09	3.66 \pm	0.81
La	0.03 \pm	0.01	0.03 \pm	0.01	0.04 \pm	0.02	0.04 \pm	0.02
Li	0.30 \pm	0.04	0.30 \pm	0.05	0.38 \pm	0.13	0.40 \pm	0.16
Mg	10.45 \pm	1.53	10.54 \pm	1.30	14.03 \pm	4.99	12.75 \pm	3.22
Mn	27.07 \pm	25.40	25.79 \pm	17.47	7.72 \pm	4.64	8.52 \pm	6.17
Mo†	8.94 \pm	2.50	8.34 \pm	2.53	17.29 \pm	7.60	16.34 \pm	7.14
Na	1.68 \pm	0.45	1.75 \pm	1.16	1.77 \pm	0.86	1.39 \pm	0.69
Nb†	2.24 \pm	0.49	2.24 \pm	0.42	3.06 \pm	1.01	3.10 \pm	1.10
Ni	0.10 \pm	0.03	0.11 \pm	0.02	0.13 \pm	0.04	0.13 \pm	0.05
P	2.39 \pm	0.96	3.31 \pm	1.10	3.83 \pm	1.06	3.73 \pm	0.93
Pb	0.06 \pm	0.02	0.07 \pm	0.02	0.06 \pm	0.02	0.06 \pm	0.03
Rb	0.08 \pm	0.01	0.08 \pm	0.01	0.09 \pm	0.04	0.09 \pm	0.05
S	26.49 \pm	10.46	27.47 \pm	10.78	11.67 \pm	3.13	11.21 \pm	2.69
Sb†	2.12 \pm	0.38	2.29 \pm	0.45	2.08 \pm	0.48	2.15 \pm	0.65
Sc	0.02 \pm	0.00	0.01 \pm	0.00	0.02 \pm	0.00	0.02 \pm	0.01

Table 4.5: Average element concentration \pm standard deviations for sediment element concentrations of the emergent plant zone of the shallow lake wetlands. Concentrations are presented in $\mu\text{mol g}^{-1}$ unless indicated by † for nmol g^{-1} .

Element	Average \pm Standard Deviation							
	Bog				Isolated			
	2014		2015		2014		2015	
Ag†	0.15 \pm	0.04	0.28 \pm	0.15	0.21 \pm	0.08	0.29 \pm	0.11
Al	4.10 \pm	1.01	3.84 \pm	1.00	5.52 \pm	2.92	7.65 \pm	5.73
As	0.03 \pm	0.02	0.03 \pm	0.03	0.02 \pm	0.01	0.02 \pm	0.01
Ba	0.66 \pm	0.47	0.76 \pm	0.77	0.43 \pm	0.19	0.45 \pm	0.15
Bi†	0.31 \pm	0.09	0.27 \pm	0.12	0.29 \pm	0.11	0.30 \pm	0.16
Ca	38.41 \pm	8.64	41.02 \pm	10.73	37.05 \pm	9.32	46.95 \pm	23.07
Cd†	1.61 \pm	0.58	1.36 \pm	0.32	1.46 \pm	0.71	1.81 \pm	1.13
Ce	0.02 \pm	0.01	0.02 \pm	0.01	0.05 \pm	0.03	0.06 \pm	0.05
Co	0.02 \pm	0.01	0.02 \pm	0.01	0.02 \pm	0.01	0.02 \pm	0.01
Cr	0.05 \pm	0.01	0.05 \pm	0.00	0.08 \pm	0.03	0.10 \pm	0.05
Cs†	1.05 \pm	0.39	0.92 \pm	0.23	1.29 \pm	0.62	1.65 \pm	1.10
Cu	0.07 \pm	0.05	0.06 \pm	0.01	0.09 \pm	0.04	0.09 \pm	0.05
Fe	6.82 \pm	3.86	8.01 \pm	4.58	6.88 \pm	2.55	8.35 \pm	3.07
Ga†	6.62 \pm	0.99	6.11 \pm	1.49	7.84 \pm	4.02	10.33 \pm	7.47
Hg†	0.32 \pm	0.06	0.36 \pm	0.04	0.25 \pm	0.07	0.26 \pm	0.04
K	3.46 \pm	1.28	3.83 \pm	2.06	4.92 \pm	2.97	4.57 \pm	2.34
La	0.01 \pm	0.00	0.01 \pm	0.00	0.02 \pm	0.02	0.03 \pm	0.03
Li	0.10 \pm	0.03	0.10 \pm	0.03	0.20 \pm	0.11	0.29 \pm	0.22
Mg	9.85 \pm	1.33	10.48 \pm	2.06	12.23 \pm	2.95	13.17 \pm	4.45
Mn	44.21 \pm	34.79	46.85 \pm	32.84	7.93 \pm	11.58	5.58 \pm	4.74
Mo†	4.23 \pm	1.39	4.36 \pm	1.95	11.75 \pm	6.72	9.65 \pm	6.00
Na	1.28 \pm	0.55	1.13 \pm	0.77	0.85 \pm	0.54	0.64 \pm	0.26
Nb†	0.95 \pm	0.28	0.88 \pm	0.26	1.68 \pm	0.95	2.28 \pm	1.62
Ni	0.04 \pm	0.01	0.04 \pm	0.01	0.06 \pm	0.03	0.07 \pm	0.04
P	2.94 \pm	0.62	3.73 \pm	0.86	3.67 \pm	0.87	3.72 \pm	0.83
Pb	0.03 \pm	0.01	0.03 \pm	0.01	0.02 \pm	0.01	0.03 \pm	0.02
Rb	0.06 \pm	0.02	0.06 \pm	0.02	0.07 \pm	0.05	0.08 \pm	0.05
S	14.41 \pm	7.79	13.11 \pm	5.39	6.59 \pm	1.98	6.56 \pm	1.99
Sb†	1.84 \pm	0.33	1.76 \pm	0.50	1.49 \pm	0.69	1.34 \pm	0.51
Sc†	8.63 \pm	2.04	8.53 \pm	1.02	11.09 \pm	4.15	13.90 \pm	6.78
Se†	13.07 \pm	1.39	10.84 \pm	1.95	8.20 \pm	2.56	7.51 \pm	1.85

Table 4.5: Average element concentration \pm standard deviations for sediment element concentrations of the emergent plant zone of the shallow lake wetlands (continued). Concentrations are presented in $\mu\text{mol g}^{-1}$ unless indicated by † for nmol g^{-1} .

Element	Average \pm Standard Deviation							
	Bog				Isolated			
	2014		2015		2014		2015	
Sr	0.44 \pm	0.11	0.44 \pm	0.10	0.21 \pm	0.08	0.24 \pm	0.06
Tl†	0.33 \pm	0.16	0.29 \pm	0.17	0.35 \pm	0.17	0.30 \pm	0.17
U†	3.21 \pm	1.49	2.91 \pm	0.98	2.92 \pm	1.80	2.67 \pm	1.53
V	0.06 \pm	0.02	0.07 \pm	0.02	0.11 \pm	0.05	0.13 \pm	0.08
Y	0.01 \pm	0.00	0.01 \pm	0.00	0.02 \pm	0.01	0.02 \pm	0.02
Zn	0.46 \pm	0.15	0.45 \pm	0.15	0.50 \pm	0.18	0.53 \pm	0.17
Zr†	4.42 \pm	2.67	4.12 \pm	2.86	7.02 \pm	4.12	8.77 \pm	6.40

Water quality parameters showed little variability with the highest variability in alkalinity and conductivity (table 4.6). Chlorophyll a was very low with highest average concentration in the bog sites of $5 \mu\text{g L}^{-1} \pm 5$. Phosphorus had the highest values in the bog sites of $0.003 \text{ mg L}^{-1} \pm 0.002$. The pH of the sites averaged around 7.7 ± 0.2 , (table 4.6). We found % OM was high for all of our sites in both the open water and emergent vegetation zones. Emergent vegetation zone had the highest % OM of 72.5% in the bog sites, and the lowest % OM was found in the open water zone in the isolated sites of 44.6% (table 4.6).

Table 4.6: Water quality parameters and sediment % OM. Averages \pm standard deviation for each parameter measured. Units for each parameter are indicated in parenthesis next to the parameter variable.

Parameter	Averages \pm Standard Deviation					
	All Sites		Bog		Isolated	
Alkalinity (mg/l)	137.26 \pm	48.18	123.51 \pm	39.51	148.90 \pm	53.17
Ammonia-nitrogen (mg/l)	0.09 \pm	0.11	0.13 \pm	0.15	0.06 \pm	0.02
Chlorophyll a (ug/l)	5.10 \pm	5.38	6.53 \pm	7.54	3.90 \pm	2.16
Conductivity (uS/cm)	293.58 \pm	102.03	261.39 \pm	79.13	320.82 \pm	113.94
Dissolved Oxygen (mg/l)	8.25 \pm	1.73	9.36 \pm	1.23	7.31 \pm	1.54
Inorganic nitrogen (mg/l)	0.07 \pm	0.09	0.05 \pm	0.00	0.10 \pm	0.12
Nitrogen (mg/l)	1.09 \pm	0.41	1.29 \pm	0.49	0.91 \pm	0.22
Nitrate and Nitrite (mg/l)	1.04 \pm	0.31	1.21 \pm	0.34	0.89 \pm	0.21
Organic Carbon (mg/l)	17.10 \pm	8.05	22.79 \pm	7.51	12.28 \pm	4.71
Ortho Phosphate (mg/l)	0.00 \pm	0.00	0.01 \pm	0.00	0.00 \pm	0.00
pH	7.74 \pm	0.22	7.73 \pm	0.20	7.75 \pm	0.24
Phosphorus (mg/l)	0.02 \pm	0.01	0.03 \pm	0.02	0.02 \pm	0.01
Sulfate (mg/l)	0.32 \pm	0.25	0.23 \pm	0.21	0.37 \pm	0.26
Temperature ($^{\circ}$ C)	10.15 \pm	1.82	10.24	1.37	10.08 \pm	2.19
Turbidity (NTU)	4.09 \pm	5.19	6.15 \pm	7.23	2.36 \pm	1.02
Sediment						
% OM Open Water	47.3 \pm	14.0	49.9 \pm	10.9	44.6 \pm	17.1
% OM Emergent Zone	68.2 \pm	15.7	72.5 \pm	7.7	63.9 \pm	23.7

NMS was used for each of the different zones of the wetland comparing plant community (main matrix) with the sediment chemistry (second matrix) for open water sediment, SAV, emergent sediment and emergent vegetation. NMS of the open water sediment and SAV data identified three axes solution to be the best, with a stress of 17.1, and a cumulative r^2 of 0.759 for all three axes. Axis one explained 40.1% of the variation, axis two explaining 20.2% and axis three explaining 15.5% (fig. 4.5). *Stuckenia pectinatus*, *Potamogeton zosteriformis*, *Utricularia vulgaris*, *Brasenia schreberi*, correlated positively with the first axis but none of the elements. *Vallisneria americana* was the only plant species to correlate negatively with the first

axis, as did Mg, indicating a positive relationship between *V. americana* and Mg concentrations. Another 15 elements correlated positively with the second axis and 5 with the third (table 4.7). Those species and elements that correlated in the same direction on the same axis show a positive relationship while those which correlate in opposite directions show a negative relationship. No plants correlated with the second axis and only four correlated with the third (table 4.7). For the NMS analysis of the emergent zone sediment and emergent plant species we found a three axis solution to be the best, with a stress of 13.4, and a cumulative r^2 of 0.870, with 42.8% of the variation explained by axis one, 25.8% and 18.4% explained by axis two and three respectively (fig. 4.6). Most elements correlated with the first and second axes, while few plants correlated with all three axes (table 4.8).

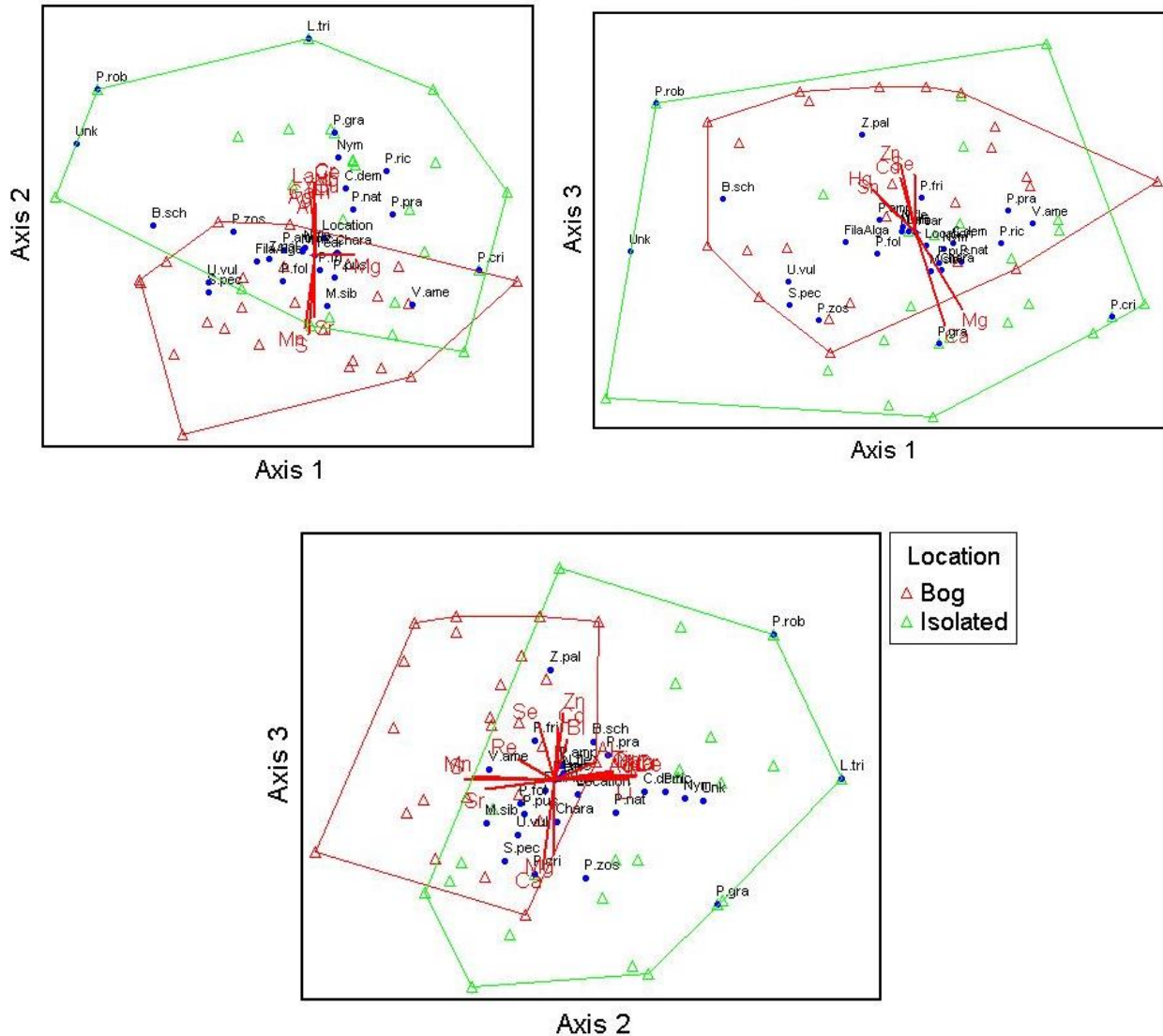


Figure 4.5: NMS ordination of SAV with a biplot of open water sediment elements. Three dimensional analysis was recommended with a cumulative r^2 value of 0.758 for all 3 axes. Axis one explained 40.1% of the variation, axis two explaining 20.2% and axis three explaining 15.5%. Convex hulls of the location are outlined in the data. Site locations are represented in the triangles.

Table 4.7: R values of axis correlations of SAV and open water sediment, found using NMS ordination methods. SAV species name are full scientific names, and elements are represented with their atomic symbol.

Species Name	Axis 1	Axis 2	Axis 3
<i>Brasenia schreberi</i>	0.648		
<i>Chara</i> spp.			0.656
<i>Myriophyllum sibiricum</i>	0.599		
<i>Nymphaea alba</i>	-0.785		
<i>Potamogeton zosteriformis</i>			0.483
<i>Stuckenia pectinatus</i>	0.503		
<i>Utricularia vulgaris</i>	0.700		
<i>Vallisneria americana</i>	-0.612		
<i>Zizania palustris</i>			-0.621
Ca			0.594
Ce		-0.559	
Cr		-0.564	
Cu		-0.503	
Ga		-0.475	
La		-0.544	
Li		-0.485	
Mg			0.536
Mn		0.563	
Nb		-0.534	
S		0.589	
Sr		0.518	
Ti		-0.477	
V		0.518	
Y		-0.502	
Zn			-0.500

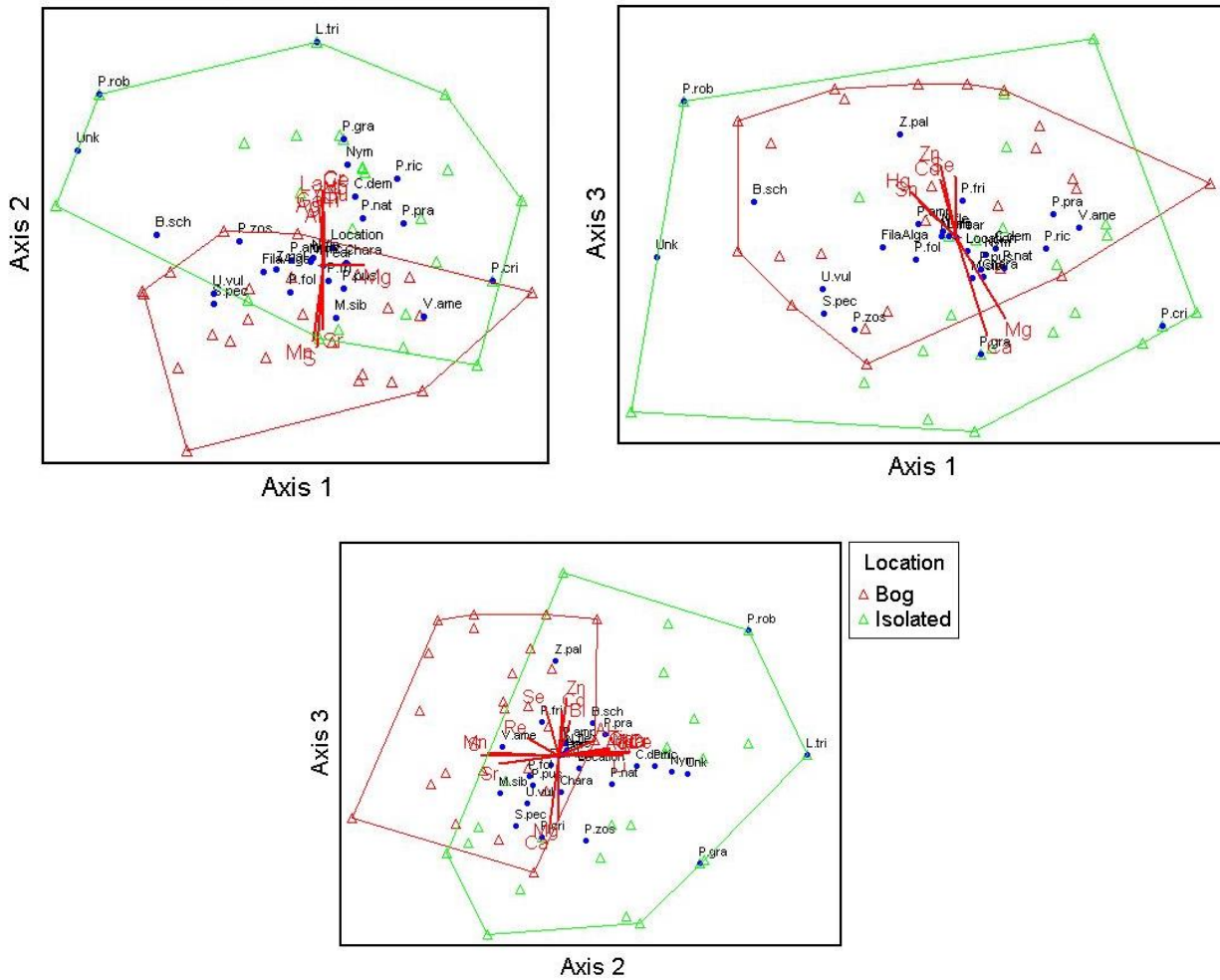


Figure 4.6: NMS ordination of emergent vegetation with a biplot of emergent sediment elements. Three dimensional analysis was recommended with a cumulative r^2 value of 0.870 for all 3 axes with 42.8% of the variation explained by axis one, 25.8% and 18.4% explained by axis two and three. Convex hulls of the location are outlined in the data. Site locations are represented in the triangles.

Table 4.8: R values of axis correlations of emergent plants and emergent sediment, found using NMS ordination methods. Plant species name are full scientific names, and elements are represented with their atomic symbol.

Species	Axis 1	Axis 2	Axis 3
<i>Athyrium filix-femina</i>	0.804		
<i>Carex retrorsa</i>			0.720
<i>Carex</i> spp.			-0.684
<i>Eleocharis</i> spp.	-0.519	0.603	
<i>Leersia oryzoides</i>	-0.512		
<i>Lysimachia</i> spp.			0.662
<i>Potentilla palustris</i>	0.484		
Al	-0.666		
Cd	-0.624		
Ce	-0.638	0.503	
Co	-0.635		
Cr	-0.614	0.592	
Cs	-0.709		
Cu	-0.556	0.538	
Ga	-0.630		
K	0.524		
La	-0.628	0.497	
Li	-0.651	0.514	
Mn		-0.639	
Na		-0.481	
Nb	-0.627		
Ni	-0.649		
Pb	-0.591		
S		-0.623	
Sc	-0.547	0.554	
Sr		-0.626	
U	-0.552		
V		0.580	
Y	-0.659		
Zr	-0.589		

Discussion

We observed low element concentrations when compared to other studies on impacted shallow lakes within Minnesota (Kissoon et al., 2015). This may explain why the presence and densities of few plants correlated with element concentrations, because the ranges of concentrations of elements in the sediment were not large enough to influence plant community composition. In a setting with no discernible inputs from outside the system, natural or anthropogenic, such as in the shallow lakes studied here, elements are available for uptake by plants from processes within the system, such as weathering of parent rock material, rainfall, and nutrient cycling. Outside influences such as runoff are not present here, which is likely to explain why element concentrations are lower compared to sites studied in the region by Kissoon et al. (2015) and Zimmer et al. (2009). Kissoon et al. (2015) examined sediment chemistry in the open water zone of shallow lakes across 2 ecoregions of Minnesota, one of those, the Laurentian mixed province is the same ecoregion in which Red Lake Nation is located. Kissoon et al. (2015) found Ca to have the highest concentrations at 1127 mmol g^{-1} within the Laurentian mixed forest ecoregion. Our study also found Ca as one of the elements with the highest concentrations but the highest average value was $130 \text{ } \mu\text{mol g}^{-1}$ in the isolated sites in 2014, much lower than Kissoon et al. (2015). Zimmer et al. (2003) found *Chara* to have a positive relationship with the N:P ratio and a negative relationship with P, while P and turbidity correlated positively. This indicated *Chara* spp. species are associated with clear lakes (those with low P levels) and high Ca concentrations. Kissoon et al. (2013) also found *Chara* spp. to be an indicator species for the Laurentian mixed forest ecoregion.

The occurrence of *Chara* spp. also had a positive relationship with concentrations Mg and a negative relationship with Zn. We compared our correlations of *Chara* with Ca and Mg,

and *Potamogeton zosteriformis* with Ca, Mg with the same element and plant data from Kissoon et al. (2015) to see if this pattern persisted over a larger gradient of conditions present. With the combined data set we found the % composition of *Chara* and Ca had a positive relationship and a Pearson's correlation coefficient of 0.20 (fig. 4.7). This indicates a weak positive relationship. We also found the relationship between % of *Chara* and Mg to have a weak negative relationship with a Pearson's correlation coefficient of -0.12 (fig. 4.8). With the combined data sets *Potamogeton zosteriformis* showed a weak positive association with Ca (Pearson's correlation coefficient 0.20) and no association with Mg (Pearson's correlation coefficient -0.01) (fig. 4.9 and fig. 4.10).

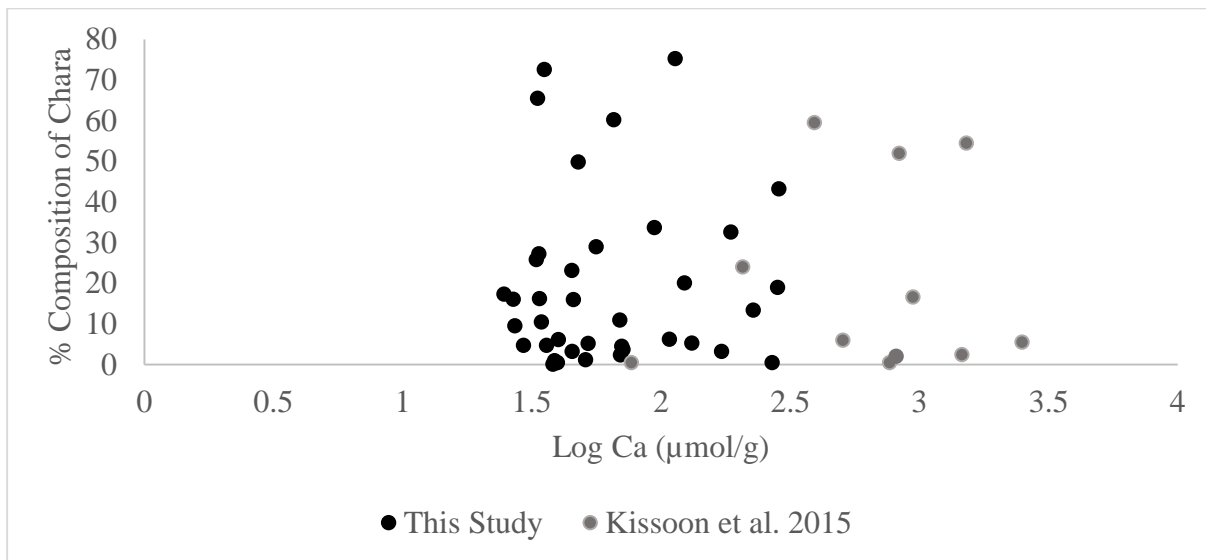


Figure 4.7: Relationship between % composition of *Chara* and Log transformed Ca ($\mu\text{mole/g}$). Values are averages per shallow lake. Pearson's correlation coefficient is 0.22, indicating a weak positive relationship.

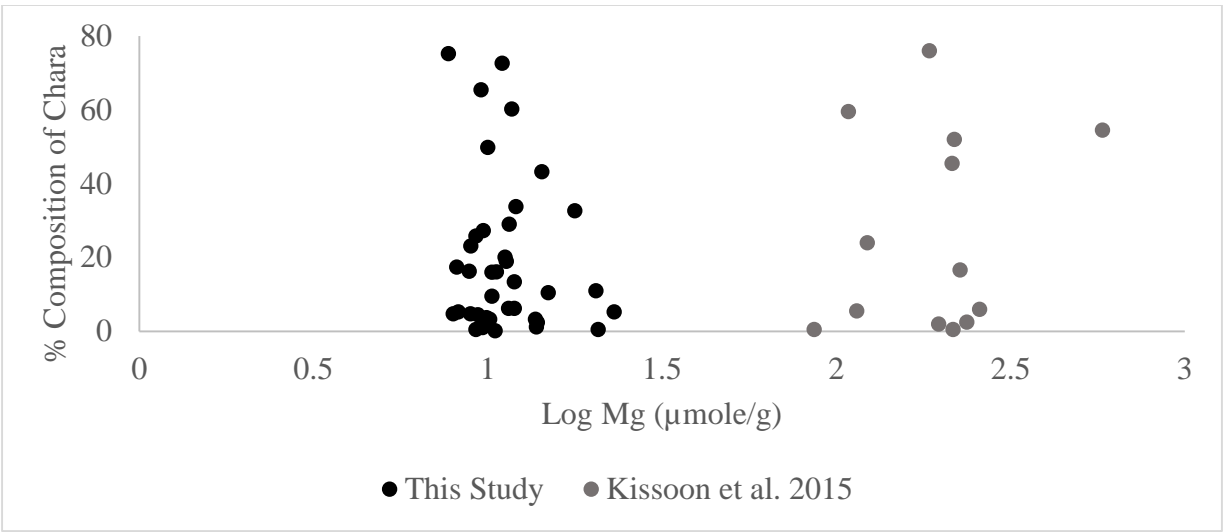


Figure 4.8: Relationship between % composition of *Chara* and Log transformed Mg ($\mu\text{mole/g}$). Values are averages per shallow lake. Pearson's correlation coefficient is 0.20, indicating a weak positive relationship.

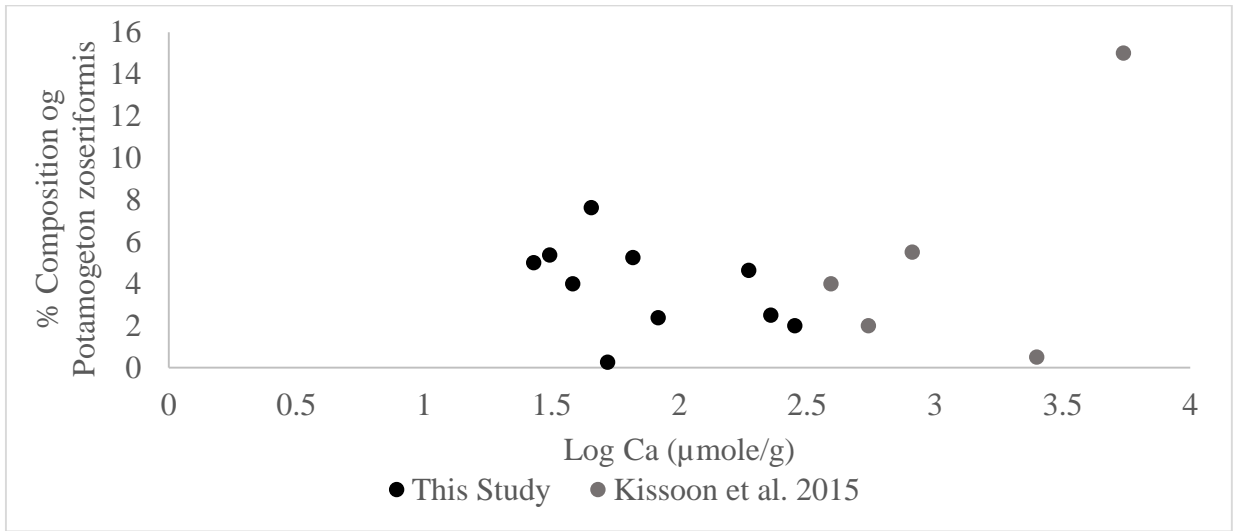


Figure 4.9: Relationship between % composition of *Potamogeton zosteriformis* and Log transformed Ca ($\mu\text{mole/g}$). Values are averages per shallow lake. Pearson's correlation coefficient is 0.64, indicating a positive relationship.

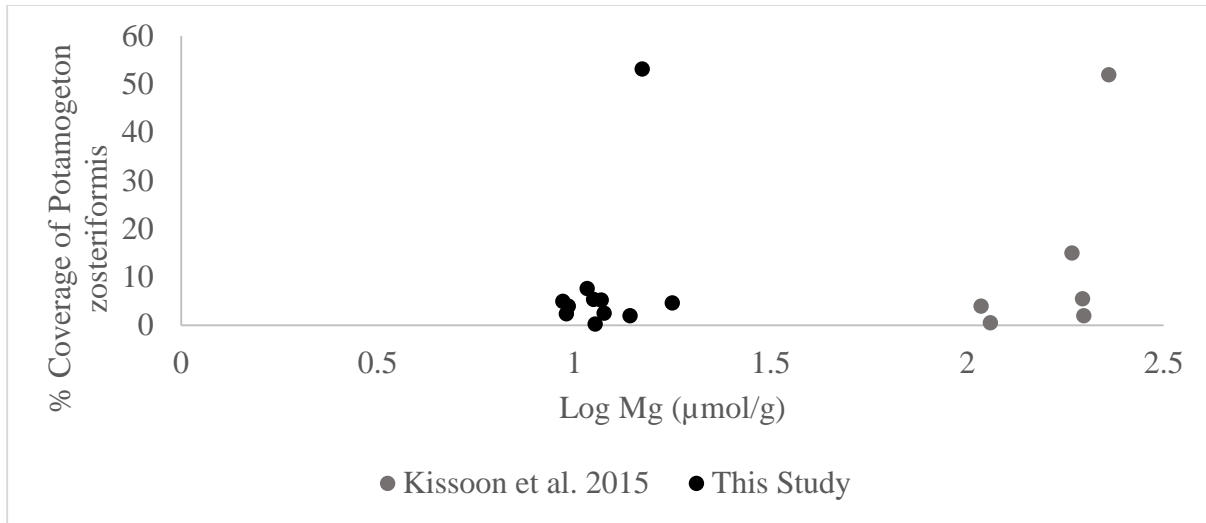


Figure 4.10: Relationship between % composition of *Potamogeton zosteriformis* and Log transformed Mg ($\mu\text{mole/g}$). Values are averages per shallow lake. Pearson's correlation coefficient is 0.28, indicating a weak positive relationship.

Both Ca and Mg are important and associated with cell wall growth (Proseus and Boyer, 2006). Mg is an essential nutrient for photosynthesis, and deficiency can result in low leaf production, production of smaller leaves, and closing of the stomata, and lower rates of photosynthesis. Therefore, smaller plants and lower primary production (Laing et al., 2000). Proseus and Boyer (2006) found that when Ca was removed from the environment the cell walls of *Chara corallina* would burst. Mg was also found to increase cell growth. *Chara* cells did not burst in the absence of Mg, but when Mg was present in the environment, cell growth was seen at higher rates than when it was absent from the environment (Proseus and Boyer, 2006). When *Chara* and *Potamogeton zosteriformis* were present in our study, it was often in large dense colonies, and would explain observed relationships of Ca, Mg, and SAV. Lakes studies by Kissoon et al. (2015) were not all in the clear state and some experienced state changes. This may also be why we did not see stronger relationships between the plant species and elements

because some lakes with high Ca and Mg concentrations may have been in the turbid un-vegetated state, leading to low % composition of the species.

Organic matter (OM) content of the soils is a factor which can also help explain element concentrations in the soil. Yellick et al. (2016) studied wetlands across North Dakota with varying conditions of disturbance by agricultural activities and found OM to explain 40% of the variation of the concentrations of elements within their data set. They also found a positive relationship between OM, which ranged between 2% and 22%, and species richness (Spearman's rank correlation index 0.415). The range in OM found by Yellick et al. (2016) is much smaller than the one in our study of 3-83%. Yellick et al. (2016) explained this by displacement of elements that do not bind to OM. The more OM within a certain volume, the higher the concentrations of elements that bind to OM, while the reverse is true for elements that have a tendency to bind to the inorganic fraction (Reimann et al., 1997). In the study by Yellick et al. (2016), this explained why S and Se showed a positive relationships with %OM, while all others showed negative relationships. The correlations, negative or positive, between some plant species and some of the element concentrations may therefore be explained by preferences of plant species for either high or low soil organic matter content. Species that prefer high organic matter content in the soils will show a negative correlation with elements that don't have high binding affinities for OM. KISSOON et al. (2015) did not find %OM to explain a significant portion of the variation within their data set of sediment element concentrations. They did find % OM to correlate negatively with many elements including Ce, Cr, Dy, Fe, Ga, Gd, La, Li, Nd, Pr, Sc, Sm, Th, V, and Y. This may explain why we didn't find more species-specific relationships with elements because we had such high %OM content in our sediment and many elements would correlate negatively with this high OM content.

Increases in element concentrations of sediments may not always indicate higher levels of primary production because some elements can be toxic to plant tissues and processes. High levels of Al and Fe can disrupt cell division and result in the loss of primary productivity and death of individuals (Ayeni et al., 2014). Different species of plants can take up elements and sequester them into tissues at different rates and can also oxidize the sediment at the root zone and increase metal mobilization into their tissues (Weis and Weis, 2004). We found *Athyrium filix-femina* and *Potentilla palustris* to be negatively associated with most elements while *Eleocharis* spp. and *Leersia oryzoides* showed positive associations with the same elements. This may indicate that *Athyrium filix-femina* and *Potentilla palustris* may be more sensitive to certain elements, and *Eleocharis* spp. and *Leersia oryzoides* may be more tolerant of a wider range of element concentrations and conditions. *Leersia hexandra* has been found in areas contaminated by metals (Pb, Zn and Cu) from mining sites indicating it has metal tolerance (Deng et al., 2006). This may indicate that metal tolerance may be a characteristic of the genus *Leersia*, which could explain why we found positive correlations with *Leersia oryzoides* and many of the elements, including metals, in our study site. Understanding the relationships between element concentrations in the substrate and plants is difficult, because it always involves multiple elements at one time (Rastetter and Shaver, 1992; Weis and Weis, 2004). We did not see more associations or stronger species specific associations between plants and elements because we were working in an un-impacted study area. We did not have sites contaminated by metal mining or pollution from industrial sources directly contributing to the element concentrations seen in the sediment. We did not have toxic levels of any of our elements seen and therefore were not able to determine relationships between toxic levels of elements and species tolerant to those levels.

Understanding sediment chemistry is important because it has the potential to influence plant community composition. Understanding plant community composition is important because high SAV biomass usually leads to clear vegetated state in shallow lake wetlands which is the desired state. Sediment element concentrations may not be limiting in a single element or unimodal manner but there may be multi-element relationships which influence individual species and community composition. Understanding what elements and plant species are present in a pristine study site will set baseline standards for wetland restoration projects, and will identify element concentrations which should be present in shallow lake wetlands and the plant communities associated with them. This will also help to better understand which element concentrations are naturally occurring in sediment of pristine study sites that have so-called background concentrations (Markert et al., 2015).

Management Implications

Future research is needed to determine if element correlations with specific plant species are indeed indicative of their need or tolerance for certain element concentrations. Laboratory experiments examining optimum levels of single and multiple element concentrations in sediment are needed to determine under what conditions plant species perform optimally. Understanding if the correlations between the plants and elements found in this study are as important as nitrogen and phosphorus associations are needed for successful restoration projects. With further understanding site specific seed mixes for restoration projects could be made based on the sediment chemistry which would yield more successful plant establishment, recruitment and regeneration of species overtime. Higher levels of SAV biomass have been associated with the clear vegetated stable state of shallow lakes which is often the desired state for shallow lake management. By first evaluating sediment chemistry and then picking the appropriate species to

plant, based on of the sediment chemistry in a shallow lake restoration projects may lead to greater plant biomass recruitment and regeneration and therefore lead to stability within the clear vegetated stable state.

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CHAPTER 5: CONCLUDING DISCUSSION

This research provides a clearer understanding of shallow lake ecology within the clear stable state, in a relatively pristine setting. Many studies have assessed shallow lake ecology, but few have done so in a pristine study site and no other studies have assessed only lakes within the clear vegetated state. This research also examined relationships between multi-element concentrations and shallow lake ecology. Very little is known about multi-element relationships and shallow lake ecology, and nothing has been known until now about multi-elements in pristine shallow lakes. We examined community characteristics of fish and plants and how they related to one another. We also evaluated if species-specific relationships existed within those communities. Many studies of shallow lake ecology have focused on the open water zone of shallow lakes, but we expanded our scope and included characterizing the emergent vegetation as well in our study. We also assess species specific relationships between sediment and plants within both the emergent vegetation and open water zones of shallow lakes.

Not all shallow lakes function equally and even in a pristine study site where all lakes were in the clear stable state, we saw differences between locations in the sediment chemistry, community composition of submerged aquatic vegetation (SAV), emergent vegetation communities, and fish communities. We found pore water element concentrations did not vary across a gradient from upland/wetland zone to the open water of the shallow lake. This indicates that pore water has the same element concentrations as the open water in our study site. This may be because nutrient and element inputs are mostly received from ground water, precipitation, and the weathering of parent rock material (Bendell-Young, 2002; Surridge et al., 2005). We also found element concentrations significantly varied over season, which could be attributed to plant senescence and changes in the activity level of the microbial community

(Frazier et al., 1996; Cronk & Fennessy, 2001). Compared to similar studies of Minnesota shallow lakes, pore water element concentrations were much lower indicating surrounding land use practices may impact element concentrations and may provide additional inputs from runoff and pollution sources (SurrIDGE et al., 2005; KISSOON et al., 2015).

Our fish communities and SAV communities contained species similar to other studies conducted in Minnesota (Zimmer et al., 2002; Zimmer et al., 2003; Herwig et al., 2010; KISSOON et al., 2015). Total fish biomass per lake was much lower than similar studies conducted in Minnesota (Zimmer et al., 2002; Zimmer et al., 2003; Herwig et al., 2010). The fish community and overall fish biomass in our study did not impact SAV community composition or total SAV biomass. This could be because fish biomass numbers were too low to have significant negative impacts on zooplankton populations and algae in the water column so the lakes in our study remained stable within the clear state (Norlin et al., 2006; Roozen et al., 2007; Nolby et al., 2015). Our sites were characterized by fish like *Ameiurus melas* (black bullhead), *Pimephales promelas* (fathead minnow), *Chrosomus eos* (northern redbelly dace), and *Perca flavescens* (yellow perch). The characteristic vegetation was *Chara* spp., *Myriophyllum sibiricum*, *Najas flexilis*, *Nuphar microphylla*, *Potamogeton amplifolius*, *Potamogeton natans*, and *Vallisneria americana*.

Multi-element concentrations in the sediment of our study were significantly lower than that of similar studies in sites with different surrounding land use and closer to human disturbances (KISSOON et al. 2015). We found few species-specific relationships between plant species and elements probably due to these low element concentrations. *Chara* spp. and *Potamogeton zosteriformis* correlated positively with Ca and Mg, *Leersia oryzoides* correlated positively with almost all of the elements while *Athyrium filix-femina* correlated negatively with

almost all of the elements. We further compared some of the species specific relationships found a clear dichotomy between the results in our study and that of Kisson et al. (2015) with *Chara* spp., *Potamogetion zosteriformis*, Ca, and Mg. This could indicate sediment element concentrations may not be limiting in a single element or unimodal manner but there may be multi-element relationships which influence individual species and community composition. Our sites also had higher organic matter when compared to similar studies which may also have influenced element concentrations and relationships with plant communities.

Stability of alternative stable states is based on positive feedback mechanisms of ecological relationships (Scheffer et al., 2001; Scheffer, 2004; Hobbs et al., 2012) (fig. 5.1). Understanding the mechanisms which drive those relationships is important in order for successful management of desired conditions and states of shallow lakes. This is also important in terms of changes in climate and anthropogenic impacts on the landscape (fig. 5.2). Knowing the mechanisms which contribute to state stability will help natural resource managers make more educated management decisions as the environment changes.

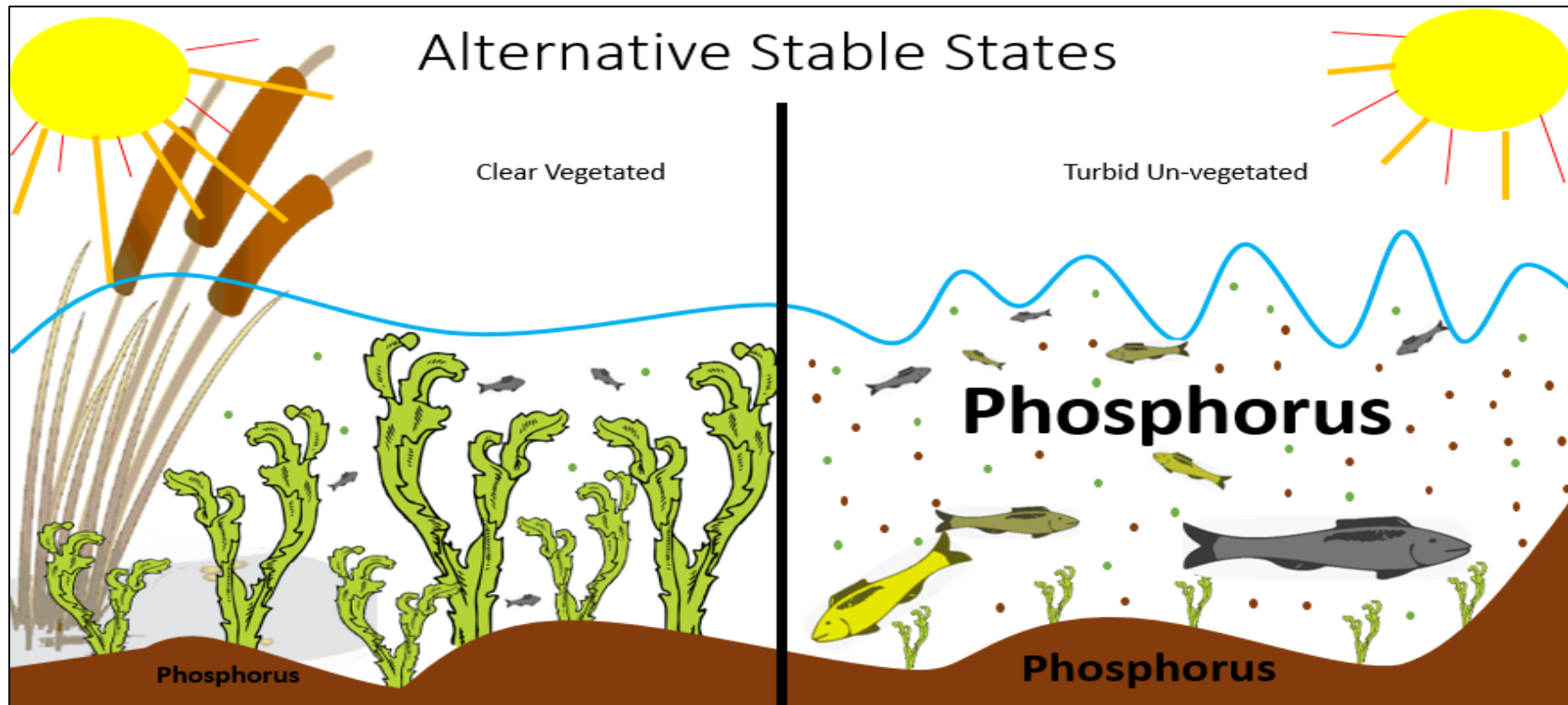


Figure 5.1: Infographic representing the characteristics of alternative stable states. On the left side is the clear vegetated state, with dense macrophytes which take up nutrients in the water column and out-compete much of the algae which keeps the water column clear. Lower fish biomass means less feeding pressure on zooplankton which in turn means more feeding pressure on phytoplankton. Macrophytes absorb wind energy and reduce resuspension of sediment particulates in the water column. A clear water column also means greater light penetration which is necessary for plant growth, as depicted on the left. The right side depicts the turbid state. Greater amounts of nutrients in the water column, such as phosphorus, lead to algal blooms. Large fish communities can re-suspend sediment particles decreasing water clarity, which in turn decreases light penetration and macrophyte growth. Without macrophytes, wind creates waves which re-suspend sediment particulates and create a more turbid water column.

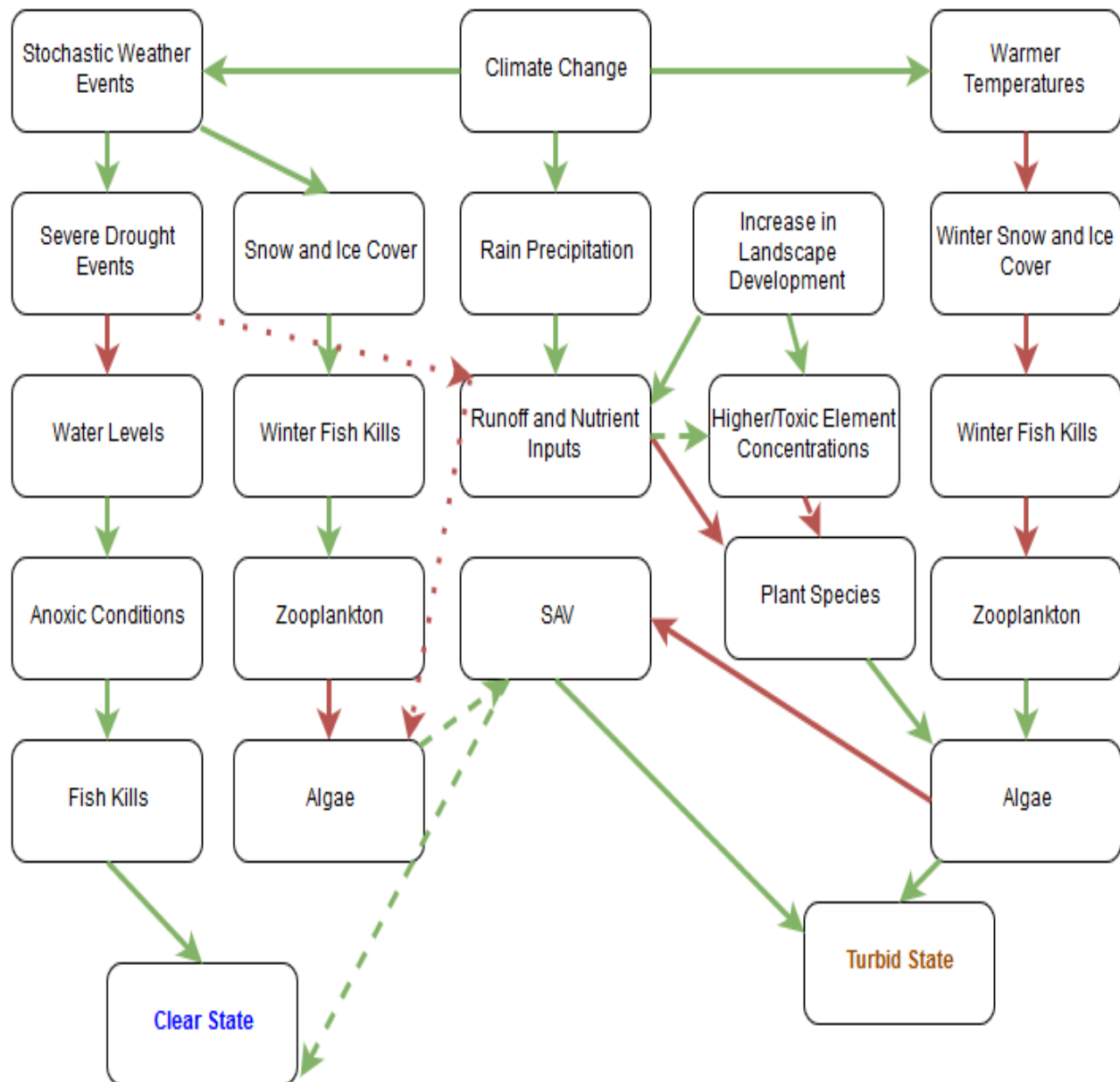


Figure 5.2: Flow diagram of potential alternative stable state outcomes of shallow lakes based on changes in environmental characteristics. Red arrows indicated decreases, green arrows indicate increase. When reading follow all the dashed arrows or solid arrows. For example, starting at climate change and working straight down; climate change could cause increases in rain precipitation which would cause increases in runoff and nutrient inputs which would cause increases in open water nutrients which would cause increases in algae, which would cause decreases in SAV and lead to increases in the probability of being in the turbid state.

The predicted climate change forecast for the Midwestern United States including Minnesota is for temperatures to increase by 3-4°C and for precipitation to increase by 6% by the year 2069 (Galatowitsch et al., 2009). These changes are also predicted to have great impacts on fish communities due to reduction in dissolved oxygen content of lake waters and rising temperatures of lake water as atmospheric temperature rises (Heinz et al., 1995). In addition, warmer temperatures can also lead to less ice and snow cover in Minnesota during the winter months (Johnson and Stefan, 2006). Less snow on top of the ice means more light penetration into the water of shallow lakes, which means planktivorous fish, visual feeders, will have an easier time finding prey. This will lead to decreases in zooplankton populations and then an increase in algae and turbidity (Sørensen et al., 2011). Sørensen et al. (2011) evaluated the effects of fish feeding pressure on zooplankton during the winter months in 2 shallow lakes and found that in enclosures with fish, the number and body size of *Daphnia* significantly decreased as compared to those enclosures without fish. They also found higher levels of Chl *a* and water turbidity in the presence of fish compared to enclosures without fish. Bramm et al. (2009) evaluated the effects of light on zooplankton predation by fish and abundance of Chl *a*. They found fish were able to significantly decrease zooplankton abundance and therefore Chl *a* increased only in the highest light exposure (100%). All other light conditions did not induce significant negative impacts to zooplankton and Chl *a* abundances. This indicates that we may not see state changes with minor changes in climate and winter weather, but we may see state changes in years of extreme winter warmth and no snow or ice cover on the shallow lakes. With overall temperatures warming though, we would expect to see fewer winter fish kills which will result in decreased zooplankton populations and increases in algae which leads to an overall trend of more turbid lakes.

Climate change can also bring an increase in stochastic weather events which do not follow projected weather trends (Johnson and Stefan, 2006). Some years may show record low temperatures and record high snow fall, or record high temperatures and record low rainfall, or any combination of extremes. Stochastic events which may include severe drought would result in lower lake water levels, increases in anoxic conditions, and fish kills, which would trend towards a clearer lake (Johnson and Stefan, 2006). Years with record low temperatures will induce greater ice cover and perhaps full freezes of shallow lakes, which will induce winter fish kills, which then will decrease predation on zooplankton allowing for an increase in zooplankton populations and a decrease in algae resulting in clearer lakes (Sørensen et al., 2011; Villena and Romo, 2007).

Increases in precipitation as the climate warms (Galatowitsch et al. 2009), will lead to perhaps increases in the number and size of wetlands on the landscape. Increases in precipitation will also lead to increases in runoff and potential increases in nutrients and element concentrations in shallow lakes from runoff. Increases in nutrients will result in increases in algae, which leads to less light penetration and then decreases in SAV which then leads to more turbid lakes (Lougheed et al., 2001; Alahuhta et al., 2012; Sahuquillo et al., 2012; Kisson et al., 2013; Wezel et al., 2013; Kisson et al., 2015). Land development can also lead to increases in runoff and nutrient accumulation in wetlands, which can have drastic effects on the plant community including species loss (Ehrenfeld and Schneider, 1991). Runoff may increase element and nutrient concentrations of pore water as well as the open water. Plants slow the movement of water through the landscape which allows for large particles to fall to the sediment and plants can take up nutrients as they settle to the root zone or in the pore water of the sediment (Mitsch and Gosselink, 2007). Increases in element and nutrient concentrations can

decrease plant species richness (Ehrenfeld and Schneider, 1991), which could result in the loss of the emergent vegetation zone of shallow lake wetlands. This in turn would increase the amount of runoff which reached the open water zone during precipitation events, increasing nutrients. This then could increase algae reducing SAV and would therefore lead to more turbid shallow lake conditions.

The mechanisms which lead to clear shallow lakes include low fish biomass per lake (Nolby et al., 2015), which can be facilitated by winter fish kills. High SAV biomass leads to a clear state because SAV can outcompete algae for essential nutrients such as nitrogen and phosphorus at low levels (P below $60\mu\text{g L}^{-1}$). Increases in rainfall (Galatowitsch et al., 2009) and human development (Ehrenfeld and Schneider, 1991) may then lead to increases in nitrogen and phosphorus in the water column which may allow for algal blooms and more turbid lake conditions. Managing for the clear state as the climate changes may need to include managing runoff as well as low fish biomass and high SAV biomass.

Understanding those ecological relationships and how they contribute to state stability within a pristine landscape provides reference points and goals for shallow lake restoration and conservation projects. As climate changes and the human population continues to expand we will expect to see changes in the environmental factors which affect shallow lake state stability. Understanding those conditions present in a pristine landscape will give natural resource managers a perspective on the ecological relationships present in clear shallow lakes. Shallow lake ecology is complex and there is still much to be learned about state stability and the driving mechanisms.

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